THE IMMUNOLOGICAL EFFECTS OF ELEMENTAL DIETS IN INFLAMMATORY BOWEL DISEASE

OBEDY MWANTEMBE B.Sc.(HB), M.B.ChB. MRCP

A thesis presented to the University of Edinburgh for the degree of Doctor of Philosophy in the Faculty of Medicine.

DECLARATION

I declare that this thesis has been composed by myself, and that the work contained within it, except on occasions which are clearly stated, was performed by myself.

OBEDY MWANTEMBE B.Sc.(HB), M.B.ChB. MRCP (UK)

SUMMARY

Inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are disorders of the gastro-intestinal system whose aetiology and pathogenesis remain unknown. It has long been known that changes in the diet can be beneficial and elemental diets (ED), devoid of polypeptide antigens, have been successfully used in some of these conditions. Thus, the aim of this thesis was to study the role of food antigens and the effect of their withdrawal on IBD immune activity by the use of ED.

Humoral immunity was studied by measuring total immunoglobulins and antibodies to: ovalbumin, ß-lactoglobulin and gliadin in serum, whole gut lavage fluid (WGLF) and parotid saliva. T cell activity was studied by measuring soluble interleukin-2 receptor in WGLF (LIL2R) and in serum (SIL2R). Macrophage activity was studied by measuring tumour necrosis factor (TNF) in WGLF and α-1 acid glycoprotein (α-1AP) in serum and WGLF. Comparisons were made between levels of these parameters in active and inactive IBD and controls. Disease activity was quantified by measuring the total concentration of immunoglobulin (Ig)G in WGLF. Patients with active IBD were prescribed ED for at least 7 days. During the course of the ED, WGLF and serum were collected prior to and after the 7th day and assayed for the above

mentioned factors.

There was no difference in serum immunoglobulins and antibodies between IBD patients and controls. Total IgM and IgG in WGLF were higher in active IBD than in controls. Food antibodies were also higher in CD, but not in UC patients compared with controls. However, there was no change in the level of immunoglobulins and food antibodies in serum, WGLF or parotid saliva in patients treated with ED, regardless of the clinical outcome. LIL2R and SIL2R were higher in active IBD compared to controls. Patients with high initial LIL2R responded to ED and showed reduction of LIL2R with improvement of disease. α-1AP was raised in all IBD (active and inactive) patients in both serum and WGLF compared to controls. α-1AP changes in IBD patients on ED were unrelated to disease response. TNF levels were higher in active IBD patients than in controls. Patients with high levels did worse on ED.

These results indicate that LIL2R levels identify those patients with predominant intestinal immune upregulation which would be amenable to antigen withdrawal. Additionally or alternatively ED may act by downregulating interleukin-2 receptors in analogue with cyclosporin treatment of IBD. We, therefore, suggest that high TNF indicates prolonged continuous upregulation of the immune activity. In this, macrophages become the prominent cells involved and are less responsive to

antigen withdrawal compared with T cells. Therefore, LIL2R and TNF can be used as markers to identify a cohort of IBD patients amenable to ED treatment. This in turn may contribute to a less empirical approach in IBD management.

ACKNOWLEDGEMENTS

I am very grateful to Professor Anne Ferguson for supervising this project, for her help towards the preparation of this thesis and for her ensuring its completion.

I would like to thank the following: Mr N. Anderson for introducing me to the practical aspects of ELISPOT; Mr. G. Brydon for the help in setting up the α-1 acid glycoprotein assay; the staff of the Animal Unit at the Western General Hospital for their assistance during my animal work; Dr C.P.C. Choudari for the collaboration in the work on the use of intestinal proteins to measure disease activity; Dr E. Arranz-Sanz for the helpful scientific discussions and very warm friendship; the Medical Illustration Department at the Western General Hospital for the preparation of figures and photographs; Dr Laura McLintock for proof reading the thesis and making very helpful comments; all the staff of the Gastrointestinal laboratories for their help as well as for providing a pleasant working environment.

I would like to thank the following consultants who gave me full access to their patients: Dr Kelvin Palmer, Dr Martin Eastwood and Mr. David Hamer-Hodges.

Finally I would like to thank Chola and Natasha for their support and patience. I am grateful to the Nestle' Foundation and the University of Edinburgh William Goodall Scholarship for providing financial assistance during my research.

INDEX

CHAPTER FIVE: ELEMENTAL DIET

CHAPTER SIX: MATERIALS AND METHODS

CHAPTER SEVEN: GUT LAVAGE FLUID PROTEINS AND DISEASE ACTIVITY IN INFLAMMATORY BOWEL DISEASE.

CHAPTER EIGHT: SECRETORY IMMUNOGLOBULIN A LEVELS IN IBD AND CONTROLS

CHAPTER TEN: HUMORAL IMMUNITY AND ELEMENTAL DIET

APPENDIX FOR CHAPTER 11

CHAPTER TWELVE: SOLUBLE INTERLEUKIN-2 RECEPTOR

APPENDIX FOR CHAPTER 12

CHAPTER THIRTEEN: TUMOUR NECROSIS FACTOR IN WHOLE GUT LAVAGE FLUID

ABBREVIATIONS

α-1AP	-	Alpha-1 acid glycoprotein
A1AT	-	Alpha-1 anti-trypsin
ASA	-	Amino-Salicylic-Acid
BIL2R	-	Serum soluble interleukin-2 receptor
BLG	-	Beta-Lactoglobulin (ß-lactoglobulin)
CDAI	-	Crohn's disease activity Index
ED	-	Elemental diet
ELISA	-	Enzyme linked immunosorbent assay
ELISPOT	-	Enzyme linked immunospot assay
GLI	-	Gliadin
IBD	-	Inflammatory bowel disease
IgA	-	Immunoglobulin A
IgE	-	Immunoglobulin E
IgG	-	Immunoglobulin G
IgM	-	Immunoglobulin M
IFN-τ	-	gamma-Interferon (IFN-τ)
IL	-	Interleukin
IL-2	-	Interleukin-2
IL-2R	-	Interleukin-2 receptor
Lavage	-	Whole gut lavage fluid
LIL2R	-	WGLF soluble interleukin-2 receptor
mRNA	-	Messenger Ribonucleic acid
M-Cell	-	Microfold/membranous cells
MHC	-	Major Histocompatibility Complex

nsIgA	-	Non-secretory IgA
OVA	-	Ovalbumin
PTI	-	Powell-Tuck index
PWM	-	Pokeweed mitogen
sIgA	-	Secretory IgA
sIL-2R	-	soluble interleukin-2 receptor
SRBC	-	Sheep red blood cells
TIgA	-	Total Immunoglobulin A
TIgG	-	Total Immunoglobulin G
TIgM	-	Total Immunoglobulin M
TNF	-	Tumour necrosis factor-α
TNF-α	-	Tumour necrosis factor-α
WGLF	-	Whole gut lavage fluid
VAS	-	Visual analogue scale

DISEASE GROUPS

aCD	-	Active Crohn's disease
aCRO	-	Active Crohn's disease
aUC	-	Active ulcerative colitis
CD	-	Crohn's disease
Con	-	Controls
CRO	-	Crohn's disease
GLTN	-	Gluten related enteropathy
inaCRO	-	Inactive Crohn's disease
inaUC	-	Inactive ulcerative colitis
UC	-	Ulcerative colitis

Addendum to ABBREVIATIONS

CFA - Complete Freund's adjuvant

CFM - Colifoam enema

CN - Colon

Cyclo - Cyclosporin

EDTA - Ethylenediamine tetracetic acid

FCS - Feotal calf serum

GRE - Gluten related enteropathy

LGLY - Lavage α-1 acid glycoprotein

LIGG - Lavage total IgG

KDa - Kilodaltons

Mptbc - Mycobacterium Paratuberculosis

NAD - No abnormality detected

NCS - Newborn calf serum

SGLY - Serum α-1 acid glycoprotein

PBS - Phosphate Buffered Saline

Pred - Prednisolone

rIFN-τ - recombinant gamma interferon

rIL-2 - recombinant IL-2

RPMI - Roswell Park Memorial Institute

SB - Small bowel

SC - Secretory piece

SFC - Spot forming cells

SLZ - Salphasalazine

PRESENTATIONS BASED ON THIS THESIS

1. Mwantembe, O., Arranz, E. and Ferguson, A. Influence of an exclusion diet on the kinetics of the systemic humoral immune response to gluten in mice. International Congress of Mucosal Immunology, Edinburgh, 1990.

2. Mwantembe, O., Choudari, C.P.C. and Ferguson, A. The role of food antibodies in inflammatory bowel disease. Presented to the Caledonian Society of Gastroenterology; Summer meeting, Aberdeen, 1991.

3. Mwantembe, O. and Ferguson, A. The immunological effects of elemental diets. Presented to the British Society of Gastroenterology; Autumn meeting, London, 1991

FOREWORD

The aetiology and pathogenesis of the Crohn's disease and ulcerative colitis remain largely unknown. Therapy is largely empirical and based mainly on the use of anti-inflammatory drugs, but there is also some evidence that chemically defined, elemental diets (ED) are effective as primary therapy. The mechanism by which such diets act is not known.

My hypothesis was that immune responses to foods are involved in some way in the pathogenesis of IBD, and that ED act by virtue of their being devoid of dietary antigens so that deleterious immune responses to these antigens are down regulated during treatment.

Our research team has developed whole gut lavage fluid (WGLF) as source of material for non-invasive research in intestinal immunity in man, and its use as a simpler yet accurate way or indice of defining disease activity in both Crohn's disease and ulcerative colitis. I participated in the early appraisal and applications of this approach.

In my first series of studies related to my main questions I used ELISA to measure antibody to three common food antigens, gliadin, ß-lactoglobulin and ovalbumin, in serum and secretions (parotid saliva and WGLF) , and enzyme-linked immunospot (ELISPOT) assay to

monitor changes in numbers of circulating antibody secreting B cells produced by withdrawal of dietary antigen.

I used these techniques to answer the following questions.

1. Do inflammatory bowel disease patients have higher food antibodies levels in serum and WGLF than controls?
2. What changes, if any, occur in food antibody levels in serum and mucosal secretions (saliva and WGLF) when patients are on ED, prescribed for purely clinical reasons?
3. Are there any differences between responders and non-responders to ED therapy?
4. Does ED lead to a fall in the numbers of antibody producing cells in peripheral circulation?

For the next phase I developed a new method for study of the cellular arm of the immune response in the gut, using WGLF, and applied this in an attempt to define the likely mechanism of immune tissue injury that is affected by ED. The markers chosen for this were IL-2 receptor to monitor T cell activity and TNF and α-1 acid glycoprotein (α-1AP) for macrophage activity.

Having established that these factors (sIL-2R, TNF and α-1AP) could be measured in WGLF as well I addressed the following questions;

1. What was the pattern of these factors in blood and whole gut lavage fluid of inflammatory bowel disease and

normal controls?

2. Within inflammatory bowel disease patients was there any difference in these factors which might explain the differences in response to elemental diet?

3. What was the pattern of these markers in responders and non-responders and what was the effect of elemental diet on the levels?

The results of this research are presented and discussed in the following chapters.

This I hope would go some way in answering a few questions about elemental diets and the immune response in inflammatory bowel disease.

CHAPTER ONE:

THE AETIOPATHOGENESIS

OF

INFLAMMATORY BOWEL DISEASE

SECTION 1A:

HISTORICAL AND CLINICO-PATHOLOGICAL PERSPECTIVES

INFLAMMATORY BOWEL DISEASE

DEFINITION

Inflammatory bowel disease (IBD) is a general term for a group of chronic inflammatory disorders of unknown aetiology involving the gastrointestinal tract. In a strict sense the diagnosis remains by exclusion as there are no pathognomonic features or specific diagnostic tests. There are two main varieties of IBD; non-specific ulcerative colitis and Crohn's disease. The diagnosis is based on the presence of typical clinical, radiographic, and pathological features.

The original description of Crohn's disease, attributed to Crohn, Ginzberg and Oppenheimer (1932), referred to disease localised to the ileum. They reported a series of patients with non-tuberculous ileal disease which they termed 'regional enteritis'. It later became evident that Crohn's disease can affect any part of the gastrointestinal tract.

The distinction between ulcerative colitis and infective colitis was first made by Samuel Wilks in 1859 though it took another half a century for ulcerative colitis to be accepted as a clinical entity in itself (Allchin, 1909). Ulcerative colitis affects mainly the large bowel.

This chapter covers briefly the general aspects, clinical and pathological aspects of IBD followed by a discussion

of the possible aetiological factors and postulated mechanisms of tissue injury in IBD.

PATHOLOGICAL AND CLINICAL FEATURES OF IBD

There are some broad similarities and distinguishing features between the two diseases (see TABLES 1:1 and 1:2). As there are no clearly defined pathognomonic features or specific diagnostic tests for either ulcerative colitis or Crohn's disease sometimes the features are insufficient to permit distinction, especially between ulcerative colitis and Crohn's colitis. This situation arises in 10-20% of such cases.

TABLE 1:1 PATHOLOGICAL FEATURES OF IBD

	ULCERATIVE COLITIS	CROHN'S DISEASE
Segmental	-	++
Transmural Involvement	-/+	++
Granulomas	-	+/++(50%)
Fibrosis	+	++
Fissuring, Fistula	-	++
Mesenteric fat, lymph node involvement	-	++

+ = 30% ++ = 60% - = < 5%

TABLE 1:2 CLINICAL FEATURES OF IBD

	ULCERATIVE COLITIS	CROHN'S DISEASE
Diarrhoea	++	++
Rectal bleeding	++	+
Abdominal pain	+	++
Palpable mass	-	++
Fistula	-	++
Strictures	-	++
Small bowel involvement	-/+ (backwash ileitis)	++
Rectal involvement	++	+/++
Perianal disease	-/+	+
Extraintestinal disease	+	+/++
Toxic megacolon	+	+/-
Recurrence after colectomy	-	+
Malignancy (with long-standing disease)	+	+/-

+ = 30% ++ = 60% - = < 5%

Crohn's disease affects any part of the gastro-intestinal system, and therefore the symptoms will depend on the part affected. As Crohn's disease affects all layers of the bowel, fistula formation, strictures and palpable

masses are more common. Ulcerative colitis primarily affects the mucosa of the colon. The granulomas in Crohn's disease are non-caseating.

AETIOLOGY

The aetiology of inflammatory bowel disease is unknown. The factors listed below may contribute to the development of IBD, and these are discussed on the following pages.

1. Genetic factors
2. Autoimmunity
3. Mucosal functional and ultrastructural abnormalities
    a. Permeability
    b. Na^+/K^+ ATPase activity
4. Psychological and environmental factors.
5. Intestinal Flora
    a. Mycobacterium
    b. Other bacteria
    c. Bacterial Constituents
6. Altered immunity to luminal antigens.
7. Inappropriate regulation of immune mediators.

Aspects of altered immune response to luminal antigens are covered in this chapter (Bacterial antigens) and also in chapter 2 as food antigens.

Inappropriate regulation of the immune mediators refers

mainly to cytokine activity and this is discussed in chapter 4.

GENETIC FACTORS

The familial incidence of IBD is 10-35% (Kirsner and Spencer, 1963). This is many times higher than the predicted rate by the prevalence of IBD in the general population. There is some evidence that the risk of IBD for females is slightly higher. The observations that IBD is more common among people of the Jewish faith (Monk et al., 1969),as well as family members and twins of affected individuals have been confirmed recently (Tysk et al., 1988). However within Israel itself the incidence of Crohn's disease (among Jews) is less than that found in the white populations of USA and Northern Europe. Ashkenazi Jews in Israel born in Europe or America are particularly prone to Crohn's disease whilst Israel born or non-Ashkenazi Jews are less susceptible (Rozen et al., 1979). These reports would suggest that though genetic factors may be important environment also plays a role.
Several rare genetic syndromes such as Turner's syndrome and subjects with Hermansky-Pudlak syndrome show an increased incidence of IBD (Shanahan et al., 1988).
There has been no clear association of IBD with HLA-A,B,C and DR antigens (Smolen et al., 1982). Extensive analysis and summation of different studies showed an increased

association in Caucasians of Crohn's disease with HLA-DR2 and ulcerative colitis with HLA B27 and HLA Cw35. In the Japanese UC was increasingly associated with HLA B5 (Biemond et al., 1986). An association between Crohn's disease and HLA-DR4 has also been reported among the Japanese (Fujita et al., 1984).

The lack of a clear pattern of association could be explained on polygenic influence, incomplete or variable penetrance or difficulty with diagnosis in the less clear cut varieties. Linkage testing, sibpair analysis suggests that Crohn's disease and ulcerative colitis are not single diseases, and that there are subsets of patients who can be defined by a combination of serological markers and molecular methods showing HLA linkage, and that other subsets exist where this does not apply. This would be in keeping with the findings of Lewkonia and McConnell (1976) who concluded, in family studies, that UC and CD have a polygenic basis and that CD would develop in individuals with many of the relevant genes while UC would occur in people with few of these genes.

AUTOIMMUNITY

Broberger and Perlmann (1962) described anticolon antibodies directed against gastrointestinal epithelial cells which also cross-reacted with Escherichia coli (E. coli) O:14. These antibodies were present in the sera

from 56% of patients with UC and 67% of patients with CD. Further work, however, proved conclusively that this was not specific to IBD patients (Carlsson et al., 1977). Antilymphocytic antibodies occurred in approximately 40% of IBD patients, 40% of household contacts, 50% of spouses and 34% of relatives of patients with IBD (Korsmeyer et al., 1975). As these antibodies reacted against combinations of DRW types (reviewed in MacDermott and Stenson, 1988) it is possible that sensitization to DR antigens may have been involved in their development. The high prevalence of these antibodies in unaffected spouses makes the role of these antibodies less clear in IBD.

More recently antibodies specific for a colonic epithelial Mr 40,000 protein have been described in sera of patients with ulcerative colitis but not in the sera of patients with Crohn's disease (Takahashi and Das, 1985). This protein is located exclusively in the colonic epithelial cells mainly along the baso-lateral domains of the plasma membrane. It is present in normal and diseased colon but its antibodies are found only in ulcerative colitis. Mr 40,000 is also present in RPMI 4788 colon cancer cells. The antibody dependent cell-mediated cytolysis by UC sera against RPMI 4788 cells was inhibited by anti-Mr 40,000 polyclonal antibody. This suggested that Mr 40,000 protein and its antibody may be involved in an autoimmune reaction that is important in

the pathogenesis of ulcerative colitis.

MUCOSAL FUNCTIONAL AND ULTRA-STRUCTURAL ABNORMALITIES

PERMEABILITY

Crohn's disease patients with small bowel disease but not those with colitis have increased absorption of orally administered ^{51}Cr-EDTA (Katz et al., 1989) compared with healthy relatives and healthy controls. However when administered by enema ^{51}Cr-EDTA is absorbed in higher amounts from patients with proctitis or distal colitis (O'Morain et al., 1986). It is equally probable that this may be a secondary phenomenon caused by mucosal inflammation leading to a breakdown in the mucosal barrier.

ENZYME ACTIVITY

Rectal mucosal sodium and potassium ATPase activity in severe ulcerative proctitis is less than in controls. In ulcerative colitis patients with or without rectal inflammation and in Crohn's colitis this enzyme activity is not significantly different from that found in controls. The reduced ATPase activity in severe ulcerative proctitis is probably secondary to the severe inflammation (Edjerham et al., 1989).

PSYCHOLOGICAL AND ENVIRONMENTAL FACTORS

Inflammatory bowel disease patients, both those previously diagnosed and those at diagnosis were found to have a higher prevalence of neuroticism and introversion than controls (Robertson et al., 1989).

Non-smokers have been shown to have an increased risk of developing UC than smokers (Lorusso et al., 1989); whereas Crohn's disease patients are more likely to be smokers (Silverstein et al., 1989).

Environment may also play a part as shown by the reported higher incidence of IBD in Afro-Caribbean and Indian children living in the UK as compared to their immigrant parents (Walker-Smith et al., 1986). A change in life style such as diet might play an important role in this increased incidence in these children though no definite factors have been established yet. However the fact that it is the offspring of the immigrants other than the immigrants themselves who show a higher incidence suggests that early and prolonged exposure to the factor(s) may be important.

INTESTINAL FLORA

Some species of the intestinal microflora (Ruseler-van et al., 1989) or their products (Juhlin, Krause and Shelley, 1980) may induce and maintain IBD by causing an

unrestrained normal or abnormal immunological reaction to themselves or unknown antigen(s). These antigens may be persistent or ubiquitous (or both).

INTRODUCTION

In 1913, Dalziel described cases of what was then termed chronic interstitial enteritis. He had the impression that the enteritis was tuberculous , "even though", he commented, "the uniform character of the affliction made it evidently not so". Repeated microscopy and histology was negative for mycobacteria. Comparatively the histology closely resembled Johne's disease, a chronic bacterial enteritis of cattle which was called pseudo-tuberculous.

It was the absence of detectable mycobacteria that led Crohn, Ginzberg and Oppenheimer (1932) to distinguish and describe CD as different from tuberculosis. Grossly and histologically Crohn's disease is very similar to mycobacterial, Yersinia or chlamydial infections. Ulcerative colitis is also very similar to Campylobacter jejuni, Shigella and Cytomegalovirus colitis.

MYCOBACTERIA AND IBD

A number of workers have looked for many strains of mycobacteria and mycobacterial antigens in Crohn's

disease with varying degrees of success. Burnman and Lennard-Jones (1978) reported the isolation of Mycobacterium kansasii from mesenteric lymph nodes of Crohn's disease patients. Das and colleagues (1988) detected Mycobacterium avium antigens in fresh frozen sections of gut wall from patients with Crohn's disease. Several recent reports suggest a role for Mycobacterium pseudoparatuberculosis (Mptbc) (Chiodin et al., 1984; 1989; Coloe et al.,1986; Haagsma et al., 1989; Gitnick et al., 1989).

There have also been a number of negative reports regarding the involvement of Mptbc in CD (Stanford et al., 1988; Graham et al., 1987;) Negative reports are also important as the tendency among most workers is not to report negative results. What is more striking about Mptbc is that it has only been isolated in patients with Crohn's disease and not those with ulcerative colitis.

Antimycobacterial therapeutic trials in Crohn's disease

Therapeutic trials as used in confirming diagnosis of difficult cases of mycobacterium tuberculosis have yielded conflicting results. Thayer et al. (1988) reported promising results in an open trial when they treated Crohn's disease patients with a combination of streptomycin and rifabutin for 4 to 6 months. There have also been reports of successful treatment of Crohn's

uveitis with the addition of rifampicin to steroid therapy and the withdrawal of rifampicin led to a recrudescence of uveitis (Wirostko, Johnson and Wirostko, 1987). Other workers (Shaffer et al., 1984; Rutgeerts et al., 1988) have not had any success with therapeutic trials. These poor therapeutic responses must not be overinterpreted, as even bacteriologically proven Mycobacterium tuberculosis infections of the intestines respond poorly to medical therapy.

Explanations for the negative results include the fact that gut inflammation could be associated with infection by cell wall deficient forms of Mycobacteria ('spheroplasts') or Mycobacteria in very small fragments such as 'Much's granules' in small areas and in small numbers (Stanford et al., 1988) or indeed just persistent mycobacterial antigens.

The response to appropriate antibiotics, as stated above, has been at best inconclusive if not overtly disappointing. In addition, the improvement in disease activity with immunosuppressive drugs (steroids, 6-Mercaptopurine, Azathioprine) does not support the idea of infection; neither does the dramatic improvement reported in a Crohn's disease patient who developed acquired immune deficiency syndrome (AIDS) (James, 1988).

OTHER BACTERIA

Burke and Axon (1988) in Leeds have shown that ulcerative colitis patients are more likely to harbour E.coli expressing adhesins than controls. Many bacteria that cause intestinal disease adhere to the gut mucosa and the adhesion of pathogenic E.coli is resistant to D-mannose. The adhesive properties of the isolates from patients with IBD were similar to those of pathogenic intestinal E.coli and there was a similar proportion of adhesive isolates in patients with infective diarrhoea due to other bacteria. This suggested that these adhesive E.coli may be of primary importance rather than arising secondarily. Reports of clinical improvements of acute UC patients when treated with tobramycin which is active against this adhesive strain of E.coli increased interest in this organism as a potential trigger of IBD. Vancomycin and metronidazole which have different spectra of activity proved as ineffective as placebo (Burke et al., 1990).

Barnes et al., (1990) reported that Crohn's disease patients had higher than control levels of antibodies to Saccharamyces cerevisiae (Baker's yeast) which correspond with disease activity . These workers suggested that this Baker's yeast may be important in the aetiology or exacerbation of Crohn's disease.

Carrageenan, a product of red seaweed used as an

emulsifier in food products such as chocolate, milk and toothpaste, has been found to cause ulcerative colitis like lesions in the guinea pig when given orally. This experimental ulcerative colitis only occurs in the presence of endogenous flora especially Bacteroides vulgatus (Onderdonk, Bronson and Cisneros, 1987). This may be a species-specific phenomenon as the combination of these two factors has not been reported to cause ulcerative colitis in man. Such reports suggest however that a different combination of substances and bacteria may apply in the case of the human disease.

Crohn's disease patients have a higher anaerobic bacteria load including Bacteriodes species (especially B.vulgatus), Eubacterium and peptostreptococci than healthy controls. It is also noteworthy that recurrences of active Crohn's disease occur consistently proximal to the ileocolic anastomosis post resection. This is invariably the area with higher microflora counts.

Pouchitis occurring after total colectomy in patients with UC appears to be related to overgrowth of anaerobic bacteria as metronidazole is frequently effective. In contrast pouchitis is virtually non-existent after total colectomy for familial polyposis (Becker and Raymond, 1986).

BACTERIAL CONSTITUENTS.

Direct injury to the gut could result from toxic cell wall polymers produced by certain species of Eubacteria (Wensick et al., 1983). Both commensal and pathogenic bacteria produce potent inflammatory molecules such as lipopolysaccharide (LPS), peptidoglycan-polysaccharide (PG-PS) and N-met-peptides including formly-methyl-leucyl-peptides (Chadwick et al., 1988).
Lipopolysaccharide and PG-PS are among the most active inducers of IL-1, TNF and other cyokines. These bacterial products work synergistically, LPS primes macrophages and polymorphonucleuocytes for triggering by f-met peptides and LPS, while heterologous PG-PS or LPS reactivates PG-PS induced inflammation (reviewed by Sartor, 1989).

IMMUNOPATHOGENESIS

Once immune activation has taken place established effector immune mechanisms of tissue injury may come into play. These are discussed below.

MECHANISMS OF TISSUE INJURY

ANTIBODY MEDIATED ENTEROPATHY

There is no evidence that IgE antibodies are important in IBD immunopathology. Circulating IgE levels in IBD are no different from healthy controls (Kruis et al., 1981).
Immune mediated cytotoxicity may be involved either directly or indirectly in IBD tissue injury.
The immune system may directly recognise and aim any of its several effector mechanisms against a particular antigen on the gut epithelium. This would apply to the Mr 40,000 protein component of human intestinal epithelium described by Takahishi and Das (1985) as discussed above. The antibody to Mr 40,000 can be adsorbed with ulcerative colitis mucosa and could therefore be involved in cytotoxicity against colonic epithelial cells. This is supported by some experiments which show that antibody dependent cell cytotoxicity may be directed against epithelial antigen (Shorter, McGill and Barn, 1984). However, this cytotoxic reaction occurs

only with colonic and not small bowel epithelial cells even in patients with Crohn's disease limited to the small intestine. Furthermore these results should be interpreted with caution as freshly isolated epithelial cells are labile and are unreliable as targets. The role of this mechanism of tissue injury remains unresolved.

The immune system may cause tissue injury indirectly. Cyotoxicity may occur when the mucosal tissue, while not being the target of the immune response itself, is damaged by an expanded inflammatory response that results from the specific antibody-antigen reaction in the lumen.

IMMUNE COMPLEXES

Immune complexes have also been implicated in the immunopathogenesis of IBD. Elevated levels of antigen-antibody complexes are found in the blood of patients with IBD (Doe et al., 1973; Richens et al., 1982). Immune complexes can activate complement and there is evidence of increased levels of activation products (Teisberg & Gjone, 1975) and increased catabolic rates of C3 and C1q in IBD (Potter et al., 1979) both indicating complement activation.

Local deposition of terminal complement complexes on intestinal epithelium has been reported in IBD (Halstensen et al., 1989).

Elmsgreen et al. (1985) studied in vitro the chemotactic

activity of peripheral blood from CD patients and their clinically unaffected first degree relatives. They observed that in a subset of the affected, 38% of patients and 18% of their relatives showed abnormal generation of chemotactic activity and decreased utilization of C3 by the alternative complement pathway. The proposal by Wakefield and colleagues (1989) that multifocal gastrointestinal infarctions mediate Crohn's disease is consistent with complement-mediated disease. Contrary to the above findings, Danis et al. (1984) suggested that the levels of circulating immune complexes in IBD are comparable to those observed in control patients with other gastrointestinal conditions such as peptic ulcers, implying that immune complexes and complement may represent a common effector mechanism rather than a primary defect.

CELL MEDIATED IMMUNITY

Cell, tissue, and cytokine studies have indicated an important role for T cells in the pathogenesis of IBD. T cell activation with anti-CD3 in organ culture of human fetal jejunum led to villous atrophy and crypt cell hyperplasia (MacDonald and Spencer, 1988), features which have been demonstrated in the mucosa adjacent to aphthous ulcers in Crohn's disease (Entrican, Busuttil and Ferguson, 1987).

The role of cellular immunity and the actions of various cytokines are discussed in chapter 4.

SUMMARY

In summary, a picture begins to emerge of IBD being dependent on the interactions between host responses, the nature and quality of antigens, the site of challenge, immunologic and genetic influences.

The probable course of events then would be as follows. Firstly that the gut mucosa is exposed to yet unknown antigen(s) (which may be microbial or dietary) and probably early in life. Due to this antigen exposure of the 'immature' immune system, or as a result of the genetic status of the individual, an enhanced immune reaction directed against the antigen is mounted. If this antigen is expressed on the host tissue or shares epitopes with the host tissue then the host tissue becomes a target of the effector mechanisms (Alun Jones et al., 1985; Takahashi and Das, 1985). On the other hand the host tissue may receive 'bystander damage' as a result of the upregulated immune response and inflammation against an unrelated antigen without itself being a direct target (Broberger and Perlmann, 1959). Failure to clear the stimulating antigen results in a persistent state of upregulation. Under this scheme differences in manifestation either as CD and UC would then depend on

the site, nature and distribution of antigen(s).

The state of upregulated immune responsiveness could then act as an adjuvant for any subsequent antigens to which the host is exposed be they dietary, microbial or viral. Hence the numerous reports of higher levels of antibodies to various antigens found in IBD sera (Taylor and Truelove, (1961); Barnes et al., 1990; Koninckx et al., 1984 and Chapter 9).

The primary trigger of the intestinal immune response could be a pathogen like the recently discovered bacterium legionella or maybe IBD is a series of diseases with some subsets induced and maintained by infection and others of different aetiologies (Mayer, 1990). The absence of specific criteria for diagnosis, which compounds the research obstacles, may be due to the possibility that in reality IBD is a spectrum of syndromes with considerable overlap in clinical symptoms, signs, endoscopic appearance and histology.

SECTION 1B:

FOOD ANTIGENS AND THE GUT

INTRODUCTION

The gastrointestinal mucosa is under constant exposure to various resident and 'traveller' antigens such as foods, bacteria, parasites and viruses. Normal mucosa absorbs food in forms and quantities able to induce an immune response (Husby et al., 1986), and perhaps higher amounts cross inflamed mucosa (Jackson et al., 1986). This immune response can take the form of a local production of secretory IgA, a systemic immune response or immunological tolerance.

ANTIGEN UPTAKE AND PRESENTATION.

In the small intestine the preferential sites for antigen uptake are the Peyer's patches. Peyer's patches have a specialised epithelium containing M (membranous) cells that transport soluble or particulate antigens intact across the epithelium to the lymphoreticular cells of the dome region of the patch. Antigen can also cross the mucosal barrier by entering lamina propria between epithelial cells (persorption), by villous uptake or active uptake via endocytosis or receptor mediated mechanisms (Nicklin, 1987).

Epithelia of Peyer's patches, like small bowel epithelia, express MHC class II molecules on the basolateral surface (Mayer et al., 1991). These class II molecules may be

involved in antigen presentation.

MUCOSAL EPITHELIAL SURFACE ANTIGENS

The demonstration of MHC class II antigens on the basolateral membranes of human and rodent intestinal enterocytes (Wiman et al., 1978; Scott et al., 1980) raises the possibility that these cells may play a part in antigen presentation. The expression of HLA class II molecules is increased in IBD (Smolen et al., 1982; Mayer et al., 1991). It is possible that this may lead to enhanced presentation of 'self' or foreign antigen. During inflammation colonic T cells release gamma interferon (IFN-γ) which induces class II MHC expression on colonic epithelial cells. This increased class II MHC expression leads to increased presentation of antigen to the local T helper cells.

Other candidate antigen presenting cells in the gut include dendritic cells, macrophages and B lymphocytes. Activated but not resting B cells can take up and present antigen in conjunction with class II MHC molecules, an ability which is also heavily dependent on the LFA-1 molecule for cell to cell adhesion (Metlay et al., 1989). As only activated B cells can sensitise T cells their role as antigen presenters is confined to the secondary responses. Dendritic cells express high levels of MHC class II products and may be involved in the primary

response.

In healthy intestinal mucosa the overall result of contact between gut mucosa and antigen leads to immune exclusion, tolerance or a limited immune reaction. On the other hand, aberrant expression of MHC class II products on epithelial cells may trigger a persistent upregulated immune inflammatory response as seen in IBD. Once T cells have been sensitised and have become memory cells, presentation of the antigen-MHC complex is enough to trigger an immune response unlike resting cells that require a second signal.

INTERACTIONS BETWEEN SYSTEMIC AND MUCOSAL IMMUNITY

Food antigens once in systemic circulation and peripheral lymph nodes are processed in the normal way and can lead to an immune response.

There is also an interaction between mucosal and systemic immune responses. T lymphocytes and B lymphocytes primed in the gut associated lymphoid tissue circulate through the systemic circulation as they disseminate to other mucosal sites. During this dissemination activated cells produce antibody as well as cytokines. Mucosal derived lymphocytes tend to home back into mucosal tissue. One factor associated with this homing property of lymphocytes is their possession of homing receptors for endothelial determinants ('vascular addresins') that are

tissue specific (Jalkanien et al., 1986).

HUMORAL IMMUNITY IN IBD

The hypothesis that there is an aberrant immune response in IBD patients is supported by the finding of higher in vitro secretion of IgG and IgM immunoglobulins by intestinal mononuclear cells of IBD patients compared to healthy controls (Macdermott et al., 1981). Baklein and Brandtzaeg (1975) reported that compared with control specimens IBD mucosa has 30 times greater numbers of IgG-containing cells and 5 times greater IgM-containing cells. In ulcerative colitis these are mainly in the IgG_1 and IgG_3 subclasses and in Crohn's disease the rise in the number of cells involves all subclasses with a predominant rise in IgG_2. Whether these changes are primary or secondary remains to be resolved. Both IgM and IgG can activate complement and this can have deleterious effects on the gut lumen. There have been a few reports of food antibodies in IBD and these are discussed below.

FOOD ANTIGENS AND ANTIBODIES IN IBD

The evidence for the involvement of diet in inflammatory bowel disease is patchy and circumstantial. Yet as early as 1771 Sir George Baker addressing the Royal College of Physicians in London gave a case report

of a patient, Thomas Wood 'A miller of Billericay' whose abdominal symptoms improved by a diet of 'sea biscuits and salt water' (Drummond and Wilbraham, 1959). Early this century the possibility that an aberrant immune response to dietary antigens caused ulcerative colitis was being seriously considered (Allchin, 1909).

Andresen (1925,1953) was among the first to mention an improvement in ulcerative colitis patients when they avoided a specific food - cow's milk. Taylor and Truelove (1961) went on to show that this was different from lactase deficiency and reported that these patients had higher serum antibody titres to milk antigens compared to normal controls. There have been many confirmatory reports of raised serum antibody titres to cow's milk proteins in UC since (Barnes et al., 1990; Lerner et al., 1989; Knoflach et al., 1987). Some workers have not found the antibody levels to food antigens to be raised (Jewell and Truelove, 1972). My work has shown that patients with Crohn's disease and not ulcerative colitis have higher antibody titres in intestinal secretions to ovalbulmin and gliadin compared to controls but there were no differences in serum antibody levels (see Chapter 9).

Ginsberg and Albert (1989) reported a case of a patient with steroid dependent Crohn's disease who responded to a milk exclusion diet with improvement in the appearance of the small intestines on X-ray. Whereas he did not react to a conventional lactose challenge, a challenge

with whole milk produced severe cramping and diarrhoea typical of his Crohn's disease. Apart from another case report by Rowe et al. (1953), in whom avoidance of milk led to marked improvement, cases linked with specific single antigens in Crohn's disease are very rare.

Reports that patients with Crohn's disease consumed more cornflakes than controls (James, 1977) have not been confirmed by other workers (Archer, 1977; Mayberry, Rhodes and Newcombe, 1978; Rawcliffe and Truelove, 1978). It has also been reported that Crohn's disease patients consume more refined sugars than healthy controls (Thornton et al., 1979) and that they also have a lower intake of fibre, fresh fruit and vegetables. These differences could be a result other than a cause of Crohn's disease. Crohn's disease patients with narrowed lumen, for example, may find refined foods which are rich in sugar and poor in fibre content less likely to cause abdominal symptoms.

Workman et al. (1984) reported that exclusion diets used in irritable bowel syndrome can be effective in Crohn's disease. The same group (Alun-Jones et al.,1985) published a list of foods that most commonly aggravate inflammatory bowel disease in which wheat and dairy products, both common western foods, were top of the list. They reported improvement in patients when these foods were excluded. The improvement in the patients reported by this group have not been reproduced in other

centres. In support of a role for wheat probably is the reported finding of significantly raised antigliadin antibodies in the sera of Crohn's disease patients compared to ulcerative colitis or controls (Koninckx et al., 1984).

Further evidence for the possible involvement of food antigens in inflammatory bowel disease is the association between early weaning and an increased incidence of UC and CD (Whorwell et al., 1979; Bergstrand & Hellers, 1983).

The proven efficacy of elemental diets in acute IBD and the prolongation of remission on exclusion diets (Jones, 1987) also adds further support to a dietary association. This is reviewed in Chapter 5.

The exact trigger and the mechanism leading to the initial immunological reactions in IBD remains unknown and the amount of absorbed antigen is not of prime importance to the magnitude of the immune response (Bruce & Ferguson, 1986). What is surprising is the paucity of any adverse effects seen that result from this constant sensitisation in the normal population. The patients with IBD may have had a breakdown in oral tolerance (reviewed in Mowat, 1987).

ORAL TOLERANCE

Oral tolerance is the state of specific immunological unresponsiveness induced by prior oral administration of antigen. Oral tolerance is easily induced with soluble antigens. Particulate antigens usually lead to systemic and local immunity other than tolerance probably as a defence mechanism against microbes (Klein and Kagnoff, 1984). As only T-dependent antigens can induce tolerance of the antibody response (Cowdery and Johlin, 1984), the characteristics of the T-cells involved are discussed below.

T CELLS IN ORAL TOLERANCE

Lymphocytes in the intestinal villous epithelium are mainly CD8+ T cells, whereas the lamina propria harbours mainly CD4+ T cells and B cells. The CD8+ suppressor T cells are involved in the induction of oral tolerance as, in the murine model, oral tolerance can be prevented by depleting suppressor T cells with cyclophosphamide or 2'-deoxyguanosine (Mowat et al., 1982; Mowat, 1986). Helper T cell anergy but not T cell clonal deletion may also play a prominent role in B cell (humoral) tolerance (Vives et al., 1980). Cell mediated and not humoral immune responses are suppressed consistently by adoptive transfer of suppressor T cells (Richman et al., 1978). In

addition suppressed DTH responses in mice fed OVA are much more easily abrogated by cyclophosphamide, to which suppressor T cells are highly susceptible, than are suppressed humoral responses (Mowat et al., 1982). Overall this can be interpreted as indicating that systemic DTH tolerance after feeding is suppressor T cell controlled while systemic antibody tolerance results from helper T cell anergy.

SUMMARY

The raised antibody levels specific for food antigens could be just one aspect of an upregulated immune response as stated in chapter 1A. Virtually all the previous work on food antibodies in IBD has been done looking at serum antibody levels which may not reflect mucosal changes (O'Mahony et al.,1991a).

One way of looking at an aetiological association between antigen and disease is antigen withdrawal during active disease and monitoring the level of immune activation markers with disease remission. This thesis addresses this problem in a variety of ways.

One approach was to look at the secretion of antibodies at the single cell level and since there is continuous traffic of lymphocytes between mucosal tissues and systemic circulation, antibody producing cells to food antigens could be enumerated in the peripheral blood by

an enzyme liked immunospot technique (ELISPOT). This method is described in chapter 6B. The other approach was to look at the levels of food antibodies in the sera of patients with IBD compared to controls and also to monitor the changes in the serum levels of food antibodies when patients were on an elemental diet. Mucosal immunity was studied by similar assays in whole gut lavage fluid (WGLF). This technique of studying mucosal immunity is discussed in chapter 6A.

CHAPTER TWO:

GASTROINTESTINAL IMMUNOGLOBULIN A ACTIVITY

SECTION 2A:

STRUCTURE, REGULATION AND FUNCTION OF IgA

INTRODUCTION: THE IMMUNOGLOBULIN A SYSTEM

Immunoglobulin A (IgA) is the most abundant immunoglobulin in the normal gastrointestinal tract (Tomasi et al., 1965). In the normal intestinal juice 95% of the IgA is secretory IgA (sIgA) with only a small amount of polymeric forms of IgA (Delacroix et al., 1982).

THE STRUCTURE OF SECRETORY IGA

Mucosal plasma cells synthesize predominantly dimeric IgA (Mestecky et al., 1983) bound together by the 'J' chain (Kutteh, Prince and Mestecky, 1982). The J chain, binds the two IgA monomers together by disulphide bonds via the Fc portion (Brandtzaeg, 1976 and 1983).

The secretory component (SC) is mainly produced by columnar cells of the crypt of LiberkÜhn in the small intestine and decreases in concentration in the epithelium covering the villi (Mostov and Blobel, 1982). Secretory component is covalently linked by disulphide bonds to one of the monomers forming the dimeric IgA (Underdown, De Rose and Plaut, 1977). There are two types of IgA, IgA1 and IgA2. There is a higher proportion of IgA2-producing cells in secretory tissues than in systemic lymphoid tissues (Kett et al.,1986).

THE REGULATION OF IgA ISOTYPE SWITCHING

There are mainly two broad theories on the mechanism of IgA isotype switching. One theory as suggested by Cebra and colleagues is that IgA isotype switch was an antigen driven process independent of other cells. This would apply in the mouse where the genes of the IgA constant domain are the most downstream. In this case repeated antigenic stimulation would progressively lead to selection of more downstream heavy chains culminating in the IgA chain (Gearhart and Cebra, 1979; Gearhart, Horwitz and Cebra, 1980).

The argument against this theory is that if the switching was sequential then IgG expressing B cells would develop into IgA expressing B cells. This is not the case, IgG B cell development is independent of IgA B cell development. Secondly whereas in mice there is only one immunoglobulin A chain at the most distal end of the immunoglobulin heavy chain gene sequence, humans have two immunoglobulin A genes, one in the middle and one at the end of the sequence (Flanagan and Rabbitts, 1982).

The other theory is that other cells (T cells or dendritic cells) control B-cell isotype switching either by direct contact or via the controlled secretion of cytokines.

Kawanishi, Salzman and Strober, (1983) showed that clonal cell populations derived from murine Peyer's

patches induced LPS activated sIgM-positive B cells to differentiate into sIgA-positive B cells. Cloned cells derived from the spleen induced differentiation into sIgG-positive B cells. They suggested that T cells from Peyer's patches induced the direct switch to the IgA isotype.

Spalding and Griffin (1986) showed that bone-marrow derived pre-B cell lines cultured with IL-3 could differentiate into IgA expressing B-cells when cultured in the presence of T cell-dendritic cell clusters derived from mucosal follicles, but not if cultured with similar clusters derived from the spleen. Dendritic cells and not T cells had to be of Peyer's patch origin for differentiation of IgA expressing B-cells. More recent work has suggests that cytokines and not cell types may control isotype switching.

Cytokines in IgA-isotype switch.

There have been reports that when transforming growth factor-ß (TGF-ß) is added to LPS-stimulated B cells there is enhanced secretion of IgA and suppressed IgM and IgG secretion (Coffman et al., 1989; Kim and Kagnoff, 1990a,b). The secretion of IgA was enhanced if IL-2 or IL-5 were present in the LPS stimulated B-cell culture (Lebman, Lee and Coffman, 1990).

Arguments against this are that these effects of TGF-ß

have only been shown under LPS and not under antigenic stimulation and only a small proportion of cells were induced to IgA synthesis. There have been no *in vivo* experiments in support of this finding and the levels of TGF-ß in mucosal follicles is similar to that in non-mucosal lymphoid tissue. The possible explanation for the high IgA in the gut is that TGF-ß is secreted in the inactive form that requires proteolytic cleavage to be activated. This is achieved in the intestinal lumen by the presence of large quantities of proteolytic enzymes of bacterial origin. Another possibility could be the requirement of intense antigenic activity such as the presence of mitogenic substances like LPS which are abundant in the gastrointestinal tract. There may be other local factors involved as these theories do not cover all other mucosal surfaces with high IgA. There is now little doubt that cytokines are involved in IgA isotype switch, although the actual mechanisms has not been elucidated.

FUNCTIONS OF COMPONENTS OF SECRETORY IgA

The role of secretory component

The secretory component is found on the basolateral surface of intestinal epithelium (Mostov and Blobel, 1982) and serves as a carrier molecule of dimeric IgA

across the epithelium into the lumen (Brandtzaeg, 1981). It also confers resistance to dimeric IgA against proteolytic enzymatic degradation (Lindh, 1975). In addition the higher proportion of IgA2 which is more resistant to bacterial proteases (Plaut, 1983) in the intestinal secretions, approximately 35% compared to 28% in serum (Jonard et al., 1984) ensures increased stability. Haneberg (1974) showed that there was still sIgA antibody activity after passage of the antibody through the infant gut.

Immunomodulation

Besides this higher resistance to proteolysis there are other features that make secretory IgA more suited to the mucosal surface. It can bind antigen without activating the classical complement cascade (Boackle, Pruitt and Mestecky, 1974). Secretory IgA does not bind to or stimulate mast cells, basophils or platelets as other classes of immunoglobulins do, though it can bind via specific receptors for the Fc-α to neutrophils, monocytes and some subpopulations of T and B cells (Fanger et al., 1980). In this way, by binding antigen in the gut lumen it can exert immune exclusion without triggering any adverse inflammatory response. Even in the presence of other classes of antibodies IgA may dampen the inflammatory response as was shown by Eddie, Schulkind

and Robbins (1971). They reported that lysis of Salmonella by serum IgM and IgG antibodies was inhibited by the addition of intestinal sIgA antibodies. They postulated that this inhibition was a result of these antibodies being replaced by sIgA via direct competition.

Immune exclusion

There is experimental evidence in animal studies that sIgA inhibits absorption of antigen (Swarbrick, Stokes and Soothill, 1979) but evidence in humans is lacking. It can be inferred, however, from the higher incidence of atopy in IgA deficient individuals (see below) that a similar mechanism applies. This must be taken with caution as loss of 'immune exclusion' capability even without reduced absorption could account for increased antigenic exposure which may be a major factor in atopic conditions.

The 'protective' role that sIgA plays is quite apparent and it would be expected that any dysregulation of this system could lead to gastrointestinal disease.

IMMUNOGLOBULIN A AND INFLAMMATORY BOWEL DISEASE (IBD)

Immunoglobulin A activity in IBD

Reports on the levels of IgA in IBD have been conflicting. Studies of immunoglobulin production and secretion *in vitro* by mononuclear cells from involved mucosa have been reported as showing diminished IgA levels compared with normal controls (Bookman and Bull, 1979; MacDermott *et al.*, 1981; Wu *et al.*, 1989). Marteau *et al.* (1990) also reported decreased levels using an *in vivo* method. The latter group collected jejunal fluid samples from 9 Crohn's disease, 5 ulcerative colitis and 11 normal controls during segmental jejunal perfusion under an occluding balloon and determined the *in vivo* jejunal secretion of polymeric IgA. They found a 50% reduction in the level of polymeric IgA secreted from normal uninvolved jejunal mucosa of Crohn's disease patients compared with normal controls or patients with ulcerative colitis. In contrast, using the same system, Jones *et al.* (1976) reported normal levels of secretory IgA in patients with Crohn's disease, though the failure to prevent contamination of jejunal fluid by saliva or pancreatic secretions was an important flaw in the design of their study.

Mucosal IgA containing cells in IBD

Immunohistochemical studies have also yielded contrasting results. Reports of high numbers of IgA-containing cells in lamina propria of the diseased mucosa have come from several workers (Baklein and Brandtzaeg, 1975 and Scott et al., 1983). The proportion of cells producing the less proteolysis resistant IgA1 has been found to be increased in the colonic mucosa of both ulcerative colitis and Crohn's disease (Kett and Brandtzaeg, 1987) as has the in vitro secretion (MacDermott et al., 1986). In IBD mucosa there is reduced expression of secretory component (Rognum et al., 1987), reduced production of J chain as well as a decrease in the capacity of mucosal IgA cells to bind cytoplasmic secretory component (Kett, Brandtzaeg and Fousa, 1988; Brandtzaeg and Korsud, 1984).

COMMENT

The different results obtained could be due to any of the following: differences between the methods employed in the studies; differences in the segments of the bowel tested, as immunoglobulin secretion rates and the numbers of mucosal plasma cells differs between different sites of the intestine (Brandtzaeg et al., 1987); or the transient changes in the level of immunoglobulins with time as reported for serum in children

(Klemola et al., 1988). However in adult populations IgA activity within individuals is more constant as shown in this study (chapter 10, Figure 10:2C). Furthermore, isolated lamina propria lymphocytes may not be representative of the proportions in the tissue. The process of cell isolation includes treatment of the tissue with various enzymes (Bookman and Bull, 1979) which might interfere with subsequent cell function. In addition when experiments are done with diseased tissue one cannot exclude the possibility that the changes observed are secondary rather than primary.

CONCLUSION

The ability of secretory IgA to bind antigen and perform many immunoglobulin functions, but not to activate complement is a very advantageous adaptive mechanism for the gut. It ensures beneficial immune activity without deleterious local inflammation. The concentrations of secretory IgA in healthy individuals and IBD patients may be related to levels of cytokines involved in the IgA synthesis. However the reports of the levels of cytokines in IBD have also not been consistent. This is discussed in chapter 4. This inconsistency could be because of the heterogenecity of IBD in terms of regional distribution of disease as well as disease activity. These factors are taken into consideration in this thesis.

In this thesis, chapter 8 examines the levels of secretory IgA and total IgA in whole gut lavage fluid (see chapter 6A, Materials and Methods) in controls and patients with IBD as well as the association between disease activity and these immunoglobulins.

SECTION 2B:

ELISPOT - A METHOD FOR SINGLE CELL SECRETORY STUDIES:

INTRODUCTION

Measurement of titres of antibody in fluids such as serum or whole gut lavage fluid does not necessarily reflect immediate secretory activity but is a sum total of accumulation of secreted and circulating antibody activity. This level is therefore influenced by the half life of the antibody, tissue deposition, inhibitors in the assay as well as the effect of clearance mechanisms such as the reticuloendothelial system. Therefore the detection and enumeration of cells secreting antibodies usually provides additional information.

HISTORY

Jerne and Nordin (1963) were the first to develop a method for studying single cell antibody secretion, the 'haemolytic plaque' assay. A similar method was described around the same time by Ingraham and Bussard (1964).
Jerne and Nordin incubated, in an agar layer, a mixture of sheep red cells (SRBC) with lymphocytes from rabbits immunised with SRBC. In the presence of guinea pig complement distinct "plaques" - areas of SRBC haemolysis appeared around lymphoid cells. They attributed this to a haemolysin produced by the individual lymphoid cells. This haemolysin, was antibody to SRBC which had fixed complement and caused the haemolysis.

The assay depended on complement for lysis therefore only cells that produced complement fixing classes of antibodies could be enumerated directly. The ELISPOT assay described below does not have these problems.

THE ELISPOT OR THE ELISA PLAQUE ASSAY

This method was described by two groups working independently (Czerkinsky et al., 1983; Sedgwick and Holt, 1983). It can be applied for detecting and enumerating single cells producing any macromolecules. Both groups applied the method initially to study antibody secreting cells.

THE PRINCIPLE

Immunoglobulin secreting cells are incubated over a plate coated with the relevant antigen. Only the immunoglobulins that are antibodies to the coated antigens are immobilised at the site of secretion. Thorough washing clears off the unbound immunoglobulins and the remaining antibodies are visualised as spots after the sequential addition of anti-Ig-alkaline phosphatase conjugate and substrate. Since the concentration of lymphocytes added is known, the proportion as well as the total number of cells secreting the particular antibody can be calculated (Figures 6B:1, 6B:2 and 6B:3, p121-122).

In essence as long as there is a purified antibody to the macromolecule in question and the cells producing the macromolecule can be induced to secrete it, *in vitro* or *in vivo* prior to plating, this technique can be applied.

ADVANTAGES AND SOME RECENT APPLICATIONS OF THE ELISPOT ASSAY.

ELISPOT is a very sensitive assay. Its sensitivity is much higher than the indirect methods of measuring antibody in the supernatant (Jannemieke *et al*., 1988; Holt *et al*., 1984). It is also more sensitive than the haemolytic plaque assay where the latter is applicable (Czerkinsky *et al*., 1983; Sedgwick and Holt, 1986).
Added to this is the fact that the plates once developed can be stored for months to years without fading as long as they are blocked with 3M NaOH.
As a result of the easy application of ELISPOT, the assay has been used to enumerate antibody-secreting cells (Held *et al*., 1989; Czerkinsiky *et al*., 1984), lymphokine secreting cells (Jannemieke and Versteegen, 1988), endotoxin secreting bacteria (Czerkinsky and Svennerholm, 1983), cells producing antibody to viruses (Lee *et al*., 1989).

SUMMARY

The ELISA-plaque assay or ELISPOT is a versatile tool for detecting and enumerating macromolecule secreting cells. As the molecules are captured at the site of secretion, the assay is not subject to dilutional or inhibitory effects. Therefore in studying the dynamics of food antibody levels with dietary manipulation, ELISPOT would be expected to detect changes earlier than ordinary ELISA (see chapter 6C). Human peripheral blood mononuclear cells have been successfully used to study intestinal antibody activity (Kantele et al., 1986; Forrest, 1988; Lycke et al., 1989 and). In this thesis ELISPOT assay was applied to detect changes in food antibody activity in relation to antigen withdrawal (chapter 10C).

CHAPTER THREE:

ALPHA-1 ACID GLYCOPROTEIN IN IBD

INTRODUCTION

Alpha-1 acid glycoproteins, sometimes called mucoproteins or seromucoids are carbohydrate-protein complexes.
Alpha-1 acid glycoprotein (α-1AP) is mainly derived from the liver as part of acute phase response (Greenspan, 1955; Werner, 1949). It is therefore probable that it can reach the gut via the bile duct.

INDUCTION OF α-1 ACID GLYCOPROTEIN

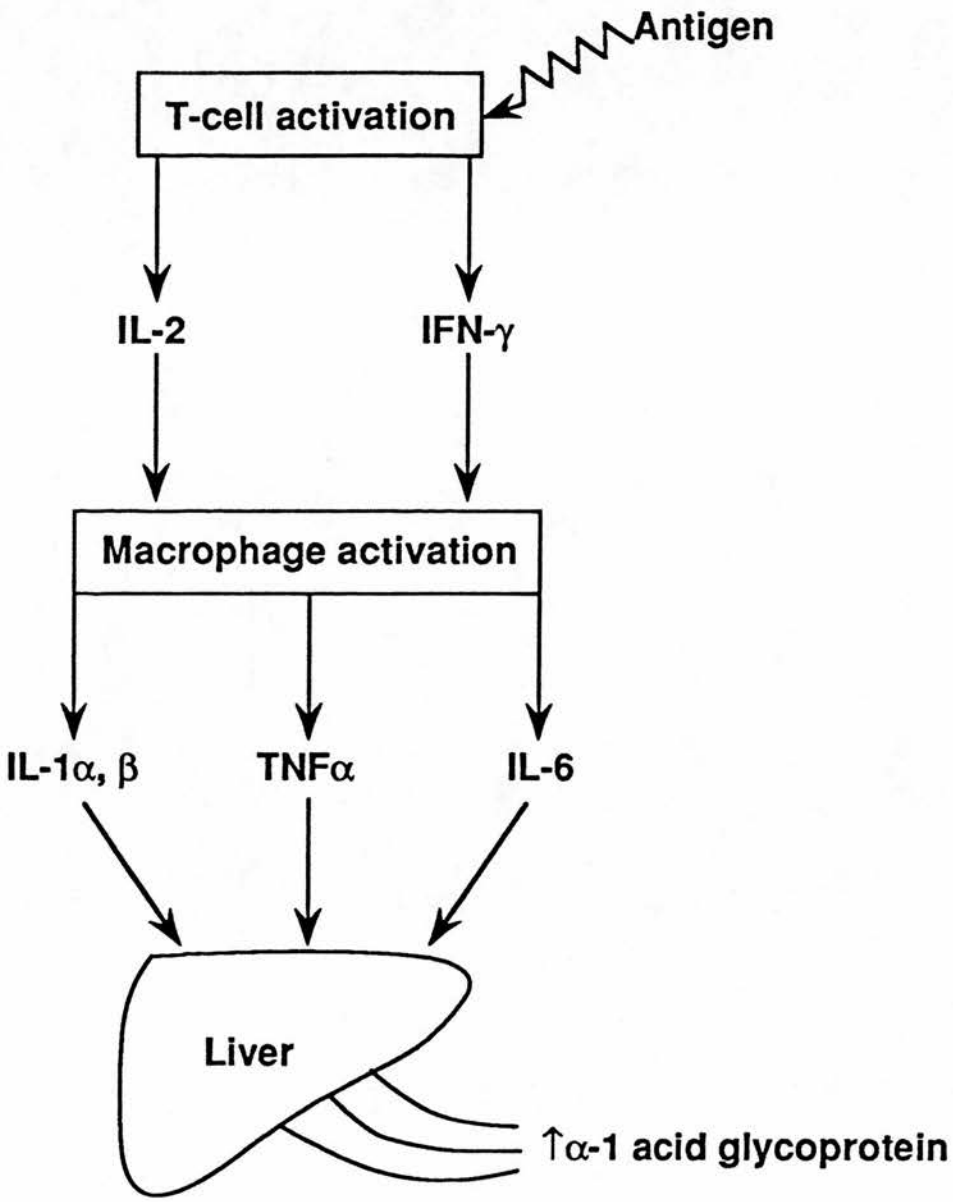
Synthesis and secretion of α-1AP is induced by the sequential activities of IL-1, IL-6 and TNF-α (Figure 3:1). Whereas many cell types can produce any one of these cytokines, in combination they are secreted mainly by activated macrophages (Emery and Salmon, 1991).

THE FUNCTION OF α-1 ACID-GLYCOPROTEIN

The exact function of α-1AP is unknown. It is an acute phase protein and is reported to be one of the most reliable indicators of acute inflammation (Peters, 1980). α-1 acid glycoprotein is increased in chronic inflammatory disease, infections and malignant conditions (Schmid, 1975). Pregnancy and intake of contraceptives decreases the levels in serum by up to 15%. Low levels have also been recorded in hypoproteinaemic states such

Figure 3:1

THE α-1 ACID GLYCOPROTEIN CHAIN
STAGES TOWARDS THE PRODUCTION OF α-1 AND GLYCOPROTEIN



When T cells are activated they produce and secrete IL-2 and IFN-γ which activate macrophages. Activated macrophages produce and secrete IL-1α and β, TNFα and IL-6, a combination which leads to increased hepatic synthesis and secretion of α-1 acid glycoprotein.

as malnutrition, nephrotic syndrome and cachexia (Peters, 1980). In addition, serum levels of α-1AP vary from day to day in an individual (Statland et al., 1976). It is not surprising therefore that there has been some debate as to what the normal values are (de la Hueuerga et al., 1956; Lockey et al., 1956; Schulze and Heremans, 1966).

ALPHA-1 ACID GLYCOPROTEIN AND INFLAMMATORY BOWEL DISEASE

There have been a few published reports suggesting that α-1AP is raised in inflammatory bowel disease (Cooke et al., 1958; Lubega et al., 1990). Cooke and colleagues (1958) studied 50 patients with Crohn's disease and 25 patients with ulcerative colitis. They reported that in Crohn's disease the level of α-1AP correlated with disease activity and was more predictive of active disease than the erythrocyte sedimentation rate. Similar observations were made for patients with ulcerative colitis. Whereas in both groups the serum level of α-1AP was higher than in normal controls or inactive disease groups only the Crohn's disease results showed a negative correlation with plasma albumin. In patients responding to treatment there was a steady fall of the level of α-1AP to normal.

Park et al. (1988) reported that as a measure of disease activity in inflammatory bowel disease, α-1AP was not impaired by the presence of malnutrition. This makes it

a particularly useful marker of disease activity in inflammatory bowel disease as it is not uncommon for these patients to be nutritionally impaired.

As a predictor of acute relapse of Crohn's disease α-1 acid orosomucoid, which comprises mainly α-1AP, was one of the two markers found to increase two to three months before an acute episode (Wright et al., 1987).

As macrophage activity is increased in IBD (Mahida et al., 1988) increased synthesis and release of IL-1, IL-6 and TNF-α may be monitored via the levels of α-1AP.

Work described in chapter 11 involved measuring the levels of α-1AP in the sera and gut secretions of patients with inflammatory bowel disease, and monitored the changes in the level in sera and intestinal secretions before and after elemental diet.

CHAPTER FOUR:

CYTOKINES

SECTION 4A:

THE INTERLEUKIN-2 SYSTEM AND IBD

General introduction

Cytokines are soluble factors which can modify the behaviour and/or growth pattern of other cells (Dexter and Moore, 1986). They are released and act typically at a purely microenvironment level. Although various cytokines are structurally very different they can have similar and overlapping effects. There is an interaction of these effects ranging from inhibition to synergism.
It is now clear that cytokines are involved in the modulation of immune responses in inflammatory bowel disease. This chapter is divided into three sections. The first two sections of this chapter review the properties of interleukin-2 and tumour necrosis factor, as these cytokines broadly cover T cell and macrophage activity. In section C cytokines are discussed with respect to the gastrointestinal tract and IBD in particular.

Introduction: The IL-2 system

In the 1960's it was thought that the immune response is controlled solely by the continued presence of antigen. This would mean that the size of the clone responding as well as the duration of the response would be governed by the magnitude and duration of antigenic stimuli. In 1965 two simultaneous reports appeared which described the first soluble mitogenic factors derived from allogeneic

mixed leukocyte culture conditioned medium (Kasakura and Lowenstein, 1965; Maclean and Gordon, 1965).

In 1970 Bach et al. reported that adherent non lymphoid accessory cells released substances that were mitogenic in culture medium. To distinguish these from lymphocyte products, Gerry and Walksman (1972) proposed the term lymphocyte activating factors (LAF) for lymphocyte products and macrophage activating factors (MAF) for products of macrophages. This section is a brief review of the lymphocye activating factor and its receptors.

Interleukin-2: Brief History

Non-antigen driven long term culture of T cells was achieved by Morgan, Roscetti and Gallo (1976) when they showed that normal human T cells could be cultured for extended periods in media that had been 'conditioned' by growth of mitogen-stimulated human peripheral mononuclear cells. After these reports a number of other groups achieved prolonged in vitro culture for cytolytic T cells to specific murine tumour antigens (Gillis and Smith, 1977a,b) and for cytolytic cells to specific human antigens (Gillis et al., 1978).

Baker et al. (1979) established that other T cells were the source of this powerful mitogenic factor and termed it T cell growth factor (TCGF). It was subsequently renamed interleukin-2 (IL-2) at the second international

lymphokine workshop (Arden et al., 1979).

The structure of IL-2

Biochemical analysis revealed that all the T cell growth promoting activity could be ascribed to a single molecule of about 15.5 kDa (Robb and Smith, 1981). With the subsequent isolation of its cDNA clone (Taniguchi et al., 1983) the molecule was fully characterized as a mature protein of 15.42 kDa encoded by four exons and separated by one short and two long introns on chromosome 4 (Fujita et al., 1983; Holbrook et al., 1984) in mice.
IL-2 can promote T cell growth only in the complete form (Ju et al., 1987) and its tertiary structure is obligatory (Yamada et al., 1987) for this action.

Interleukin-2 receptors

The structure of IL-2 receptor

High affinity IL-2 receptors (IL-2R) consist of two distinct polypeptide chains, each of which contains an IL-2 binding site (Tsudo et al., 1986; Teshigawara et al., 1987). Each molecule consists of a smaller 55kDa chain (Robb and Warner, 1983) and a larger 75kDa chain (Tsudo et al., 1987a). Teshigawara et al. (1987) called the bigger 75kDa polypeptide IL-2α and the smaller 55kDa

polypeptide chain IL-2ß to conform with the convention followed in naming receptor systems. The problem, however, was that Sharon et al. (1986) had named these receptors differently calling the 55kDa, α and the 75kDa, ß. In this thesis ß-chain will refer to the 75kDa polypeptide and α-chain to the 55kDa receptor. The 55kDa chain is also called the tac antigen (Robb, Green and Rusk, 1984).

The p75 (intermediate-affinity) receptor

Tsudo et al. 1986 reported the existence of a protein that interacted with IL-2 but did not precipitate with anti-tac. This was the 75kDa (ß) chain. The ß chain transmits the signal for T cell proliferation without a requirement for any input from the 55kDa (α) chain, and some supporting evidence for this will be reviewed.

Fujii et al. (1986) added radiolabelled IL-2 in an in vitro culture of human T cells and reported that whereas high affinity receptors (αß) were internalised after contact with IL-2, p55 chains were not internalised to any measurable extent.

Wang and Smith (1987) reported that IL-2 promoted human T cell growth and proliferation in vitro in the presence of antibody to the α-chain and at concentrations explicable only by its interaction with the ß-chain. Natural killer cells with only 75kDa chains on their surface also

respond fully to IL-2 (Tsudo et al., 1987b; Siegel et al., 1987).

The physico-chemical characteristics of IL-2 receptors

The two polypeptide chains are not connected via a covalent disulphide bond but interact via non-covalent forces to form a heterodimeric high affinity IL-2 receptor. The association is dynamic and subject to the law of mass action and dissociation. For example when anti-tac binds to the α-chain, it disrupts the α-ß heterodimer and completely prevents binding of IL-2 to both the high affinity receptor (α-ß) or the p55 chain but it has no effect on the 75kDa chain.

IL-2 binds and dissociates from the 75kDa chain slowly but it has a very rapid interaction both in terms of binding and dissociation with the 55kDa chains (Wang and Smith, 1987; Lowenthal and Green, 1987). The high affinity receptor (α-ß) derives its fast association rate from the α-chain and its slow dissociation rate from the ß-chain giving it a thousand fold increase in binding affinity compared to the individual affinities of the chains.

IL-2 and IL-2 surface receptor density

After contact with IL-2, high affinity IL-2R expression decreases. This is because the receptor-interleukin complex is endocytosed into the cell where it undergoes degradation (Fujii et al., 1986). The reduction in the surface receptor acts as a down-regulatory mechanism of the immune response. Thus unless there is continued antigen-stimulated replenishment of the receptor the immune reaction will tend to diminish secondary to the reduced high affinity IL-2R levels. This down-regulation of receptor levels is one of the mechanisms for transferring control of the immune system from external factors (foreign antigen) alone to endogenous factors such as other cytokines which depend on and reflect the state of the internal millieu. Other endogenous factors include TNF-α (Beutler and Cerami, 1988), IFN-τ (Heberman et al., 1979), IL-4 and IL-5 (Loughnan and Nossal, 1989) and IL-2 itself, all of which affect expression of IL-2R.

IL-2R synthesis

During an immune response activated T cells have increased DNA transcription and mRNA translation for the synthesis of the 55kDa chain and IL-2. The genes for these molecules have different promoter/enhancer mechanisms which means that they have to be activated

separately. It is likely that they respond to a common signal generated by the T cell receptor that subsequently bifurcates into two separate pathways promoting the IL-2 gene separately from the 55kDa chain gene (Fujita et al., 1986). There is only a modest upregulation of the 75kDa chain synthesis at the mRNA level following activation (Hatakeyama et al., 1989).

Activated B cells also express IL-2 receptors. However there are about ten times fewer IL-2 receptors as expressed by activated murine (Nakanishi et al., 1984) or human B cells than are expressed by activated T cells of the same species (Muraguchi et al., 1985), whether quantified by radiolabelled IL-2 or monoclonal antibodies to the 55kDa chain.

The T cell immune response and interleukin-2

Resting T cells neither produce IL-2 nor respond to exogenously added IL-2 (Smith et al., 1979). Antigen is required for the initial induction of T cell response moving the cell from the G_o to the G_1 stage of the cycle (Meuer et al., 1984). Once the cell has gone into the G_1 phase, expression of functional IL-2 receptors is the only other variable that ultimately determines whether the process proceeds to synthesis and cell division and how long the clonal expansion occurs after antigen stimulation (Herzberg and Smith 1987). Further

cell progression is dependent on three factors; the concentration of IL-2, the density of IL-2 receptors and the duration of contact between IL-2 and IL-2 receptors (Cantrell and Smith, 1983 and 1984). IL-2 promotes a gradual and sustained increase in cell size, the lymphocyte blastic transformation, and prepares the cell for DNA replication (Nowell, 1960). During this process the density of 55kDa may increase to 5-20 fold that of high affinity receptors (Smith and Cantrell, 1985). Some of the p55 is shed into the surrounding fluid and this has been termed cell free or soluble interleukin-2 receptor.

Cell free or soluble IL-2R

This receptor is a very weak binder of IL-2 and its function remains unknown. Some of the postulated functions of soluble IL-2 receptor (sIL-2R) are reviewed below.

One school of thought suggests that sIL-2R acts as part of a feedback loop. In this model, sIL-2R is thought to bind IL-2 before IL-2 reaches its target cells thereby blocking its physical association. The more IL-2 activated cells there are, the higher the level of sIL-2R, the higher the block (Rubin et al., 1986).

Smith and Cantrell (1985) proposed that sIL-2R down regulates the immune response by covering the high

affinity receptor sites on the cell surface, as despite an increase in the density of sIL-2R during an immune response, sIL-2R does not itself facilitate internalisation.

Others have suggested that sIL-2R facilitates the upregulation the immune response. When sIL-2 binds IL-2, the combined molecule may be stabler than IL-2 alone thereby enabling the IL-2 to be transported over a longer distance and along the cell membrane. This would increase the chance of IL-2 associating with the complete high affinity receptor (Saito et al.,1988) or 75kDa chain. Contact between this loose complex and the high-affinity cell surface receptor leads to the transfer of IL-2 to the higher affinity cell surface receptor and thereby releasing the sIL-2R to capture some more IL-2. This has been called the 'Schlepper' effect. In this way IL-2 is directed and concentrated onto the relevant receptor sites.

The interleukin-2 system and inflammatory bowel disease

There have been conflicting reports regarding IL-2 production by IBD mucosa. Fiocchi et al. (1984) reported decreased serum levels of IL-2 while Brynskov and Tvede (1990) have reported that the levels are increased in patients with IBD compared to healthy controls. These differences could be a result of different methodology

as the earlier work of Fiocchi et al. (1984) used a bioassay which may be subject to many other influences such as the presence of antibodies or/and natural inhibitors and antagonistic cytokines in the culture media. Evidence so far supports an upregulation of the IL-2 system in IBD as other reports cited below indicate. Mahida et al. (1990) reported that there was increased sIL-2R in the serum from peripheral and mesenteric vessels of patients with IBD than controls. This is supported by studies in vitro which showed that there is increased release of sIL-2R by colonic lamina propria mononuclear cells of IBD patients (Schreiber et al., 1991). Mahida et al. (1990) also observed that mesenteric sIL-2R levels were higher than levels in peripheral blood suggesting that a considerable amount of this sIL-2R was of intestinal origin. In addition operative specimens showed higher intestinal tissue IL-2R in the IBD specimens compared to controls.

Mullin et al. (1991) reported that the levels of IL-2 mRNA were increased in active Crohn's disease but not in active or inactive ulcerative colitis tissue compared to tissues from healthy controls.

Choy et al. (1990) examined intestinal tissue from children with inflammatory bowel disease and healthy controls by staining for CD4, CD8, CD3, HLA-DR and CD25. The CD25 antigen is the 55kDa surface antigen (Tac antigen) which functions as a receptor of IL-2 on

activated cells. They reported that most of the CD25+ cells in the lamina propria of active Crohn's disease were CD3+, CD4+ but CD8- (T cells) whereas the cells in the colonic mucosa of ulcerative colitis were CD3-, CD4+ and HLA-DR+ suggesting that they were not T cells but macrophages. Colonic Crohn's tissue stained for cells in the same distribution as small bowel Crohn's but the percentage of CD25+ T cells was close to that of ulcerative colitis. These findings if confirmed would go some way in aiding the distinction between Crohn's disease and ulcerative colitis in the 20% of cases where colonic Crohn's is difficult to distinguish from ulcerative colitis.

Therapeutic implications of IL-2 system

A number of commonly used immunosuppresive or immunomodulatory drugs act via the IL-2 system. For example glucocorticoids suppress the immune system through a selective inhibition of IL-2 production with reportedly very little effect on IL-2R expression (Gillis et al., 1979). In acute IBD cyclosporine has been found to act by downregulating the interleukin-2 receptor (Brynskov and Tvede, 1990).

It is therefore conceivable that the therapeutic effect of elemental diet in IBD is via the downregulation of the immune response; a down regulation in the IL-2 system may

be one of the underlying mechanisms. This may be detectable as a fall in T cell activation markers such sIL-2R. This question was addressed in this thesis (chapter 12) by measuring this marker in patients on elemental diet.

SECTION 4B:

TUMOUR NECROSIS FACTOR AND IBD

Introduction

The name tumour necrosis factor (TNF-α or TNF) originates from the old observation that intercurrent bacterial infections caused necrosis of malignant tumours (Old, 1985). Late last century, the American surgeon Coley attempted to induce tumour necrosis by treating cancer patients with broth from the culture of Streptococcus and Serratia organisms. This met with limited success partly due to sepsis (Old, 1985). The factor responsible for causing tumour necrosis was later isolated (Aggarwal et al., 1985) and purified (Beutler et al., 1985a and 1985b) and termed tumour necrosis factor (TNF).

In the mid 1970's other observations were made that rabbits infected with T. brucei, despite anorexia and a profound wasting diathesis, showed a marked hypertriglyceridaemia. This was a result of lipoprotein lipase deficiency. The factor responsible for this hypertriglyceridaemia and cachexia was called cachectin (Rouzer and Cerami, 1980). Cachectin was subsequently shown to be identical to TNF-α by serological and cloning studies and by direct comparison of biological activities (Caput et al., 1986 and Beutler and Cerami, 1986).

Secretion of TNF-α

TNF-α is mainly derived from macrophages. A variety of other cells can synthesise TNF such as natural killer cells, astrocytes, Kupffer cells , lymphocytes (Sherry and Cerami, 1988) and paneth cells (Kesha et al., 1990). The human TNF gene is located on the short arm of chromosome 6. Production and secretion of TNF can be triggered by heterogenous stimuli such as viral, fungal or parasitic antigens, toxins and cytokines such as IL-1 and IL-6 (reviewed in Beutler and Cerami, 1988).

Tumour necrosis factor and receptors

There are two forms of mature TNF; one is a cell-free variety consisting of 157 amino acids (mol. wt. 17kDa), and the other is a 26kDa transmembrane form that remains cell-associated acting at the local tissue level (Sherry and Cerami, 1988).

There are two independent cell surface receptors for TNF-α (Durum et al., 1991) with molecular weights of 55kDa and 75kDa. Both can be shed and occur in the soluble form being present in the serum of healthy individuals at surprisingly high levels (1-4ng/ml). The soluble receptor can serve firstly as a means to absorb and inactivate TNF spreading outwith the local area of inflammation, secondly as an important slow release

reservoir to maintain some background of biologically available TNF (Fomsgaard, Svenson and Bendtzen, 1989). These receptors may act as an immediate feedback mechanism to mitigate the potentially harmful effects of a sudden surge of TNF. The latter function is supported by the fact that there is rapid shedding of TNF receptors when TNF is released (Bendtzen et al., 1988).

All somatic tissue except erythrocytes possess receptors for TNF. Probably internalisation of the receptor-TNF complex is required for cell killing but not all cells with receptors are killed (reviewed by Beutler and Cerami, 1988).

Physiological and cytopathic effects of TNF in vivo

The effect of TNF on target cells include fragmentation of DNA, generation of free radicals and activation of target cell lysosomal enzymes (reviewed in Beutler and Cerami, 1988).

Tumour necrosis factor has widespread effects in vivo. It mediates endotoxic shock by promoting production of triglycerides. The hypertryglyceridaemia resulting from the inhibition of lipoprotein lipase may act as a substrate for the provision of additional energy for the upregulated immune response (Porat, 1989). Tumour necrosis results probably mainly from the effect of TNF on the vascular supply to the tumour (Carswell et al., 1975)

through its toxicity to the endothelial cells (Nawroth and Stern, 1986).

Many features that occur in IBD may also be a result of the action of TNF. Tumour necrosis factor can cause fever (Dinarello et al., 1986), the induction of acute phase proteins (Sipe et al., 1987) as well as neutrophilia (Djeu et al., 1986) features quite in keeping with acute IBD.

Growth failure and cachexia are features of IBD. Many workers have suggested that these features may also be mediated by TNF. Some of the work addressing this link is reviewed below.

Hyams et al. (1991) have reported that the level of serum TNF was not elevated in children with inflammatory bowel disease. Therefore, they suggested, it could not account for much of the growth failure in these children.

Alstead et al. (1991) found that the levels of TNF in serum was higher in inflammatory bowel disease patients with extraintestinal manifestations such as joint disease. They suggested that it may then be implicated in the pathogenesis of extragastrointestinal manifestations of inflammatory bowel disease.

Murch et al. (1991) also looked at the possible contribution of TNF to suppression of lineal growth which affects up to 30% of patients with childhood chronic inflammatory bowel disease. These workers found significantly higher serum TNF levels in children with

relapsed colonic inflammatory bowel disease than in those with small bowel Crohn's disease. This applied despite the similarities in the severity of relapse by disease indices. The colonic inflammatory bowel disease group showed a lower current growth velocity. Even within Crohn's disease the current growth velocity was significantly greater in the group with relapsed small bowel disease compared to those with relapse of Crohn's colitis. These authors while acknowledging that chronic undernutrition is the major cause of growth failure in children , stated that these marked differences in serum TNF levels between the groups also played a part in the reducing growth in the colonic group. In addition TNF per se can cause profound anorexia (Michie et al., 1989). At the cellular level TNF causes metabolic changes such as increased catabolism (Torti et al., 1985) that lead to wasting despite unlimited food intake (Oliff et al., 1987).

Source of the TNF in gastrointestinal tract

Murch et al. (1991) commented further that serum TNF in IBD may be just an overspill from local mucosa and therefore may represent only a fraction of the mucosal production. Paneth cells interspersed between epithelial cells contain TNF mRNA and they could be an important source of mucosal TNF (Kesha et al., 1990). Another

source of TNF may be activated mucosal macrophages and T cells both of which are abundant in active inflammatory bowel disease (Mahida et al., 1988). MacDonald et al. (1990) demonstrated by an ELISPOT technique that there were higher numbers of cells secreting TNF-α in the gut mucosa of IBD patients compared to healthy controls. Neguchi et al. (1991) found that the release of TNF from active colonic IBD mucosa into the medium in an in-vitro culture was significantly higher compared to that of control. Results presented in chapter 13 of this thesis show that TNF levels in whole gut lavage fluid were highest in the group with colonic inflammatory bowel disease.

The association between TNF and IBD pathology

Organ culture studies have shown that the presence of activated T cells can lead to local tissue damage of human fetal jejunum (MacDonald and Spencer, 1988) and nearly all this cytopathic effect was due to the presence of TNF (Deem, Shanahan and Targan, 1991). Tumour necrosis factor has been shown in animals to induce a cytopathic effect on bowel mucosa (Tracey et al.,1988).

The granuloma formation in Crohn's disease is probably also cytokine mediated. IL-1 (Kobayashi et al., 1985), IL-2, TNF and IFN-τ (Murray et al., 1987) have all been found in granulomas. This compares with infection with

Schistosoma mansoni where there is evidence that IL-1 is important in the initiation of granulomas in vivo while TNF is important for the maintenance of the granuloma (Chensue et al., 1989).

Kindler et al.(1989) for example have shown that the bacille Calmette-Guerin granuloma is related to the local accumulation of TNF mRNA in the granuloma. Injection of anti-TNF antibodies suppressed this granuloma formation.

Both the cytopathic effects and granuloma formation suggest a role for TNF in the immunopathogenesis of both ulcerative colitis and Crohn's disease.

On the other hand care must be taken as persistently raised TNF activity might be the mechanism by which the gut reduces the pathogenic effects of a condition it is incapable of clearing as discussed below (Section C).

SECTION 4C:

CYTOKINE INTERACTIONS IN INTESTINAL DISEASES

Introduction

A knowledge of what cytokines are involved in IBD may be of diagnostic and therapeutic benefit. It may aid distinction between less distinct cases of ulcerative colitis and Crohn's colitis (see Chapters 12 and 13). Secondly whereas other molecular mediators, such as prostaglandins, leukotrienes, platelet activating factors and oxygen free radicles may be of pathogenic importance, they mainly act at the effector end of the spectrum of inflammation. Cytokines on the other hand are involved throughout the immune response. This section reviews the literature on cytokines and the gastrointestinal tract with particular reference to inflammatory bowel disease.

Mucosal T cell function and cytokines

Intraepithelial lymphocytes proliferate poorly when stimulated with mitogen but they produce cytokines and their production of IL-2 and IFN-τ is similar to that of mitogen activated peripheral blood mononuclear cells (Ebert et al., 1990). Studies from normal non-human primates have shown that intestinal lymphocytes have increased expression of 55kDa chain (Zeitz et al., 1988). Zeitz et al. (1988) also showed that when these lamina propria T cells are cultured with recombinant IL-2R (rIL-2) their proliferative response was higher than that

of T cells from peripheral blood or mesenteric lymph nodes. The steady state level of cytokines also differs between lamina propria T lymphocytes and systemic T lymphocytes. In experiments done *in vitro* using activation by ionophore and phorbol myristate acetate, both mesenteric lymph node and lamina propria T cells expressed high levels of mRNA for IL-4 and IL-5 in comparison to cells from peripheral blood, spleen or peripheral lymph nodes (James, Kwan and Sneller, 1990). In addition lamina propria lymphocytes had the highest levels of IL-2 mRNA, whereas mesenteric lymph nodes had lower IL-2 mRNA. IFN-τ was also low in mesenteric lymph nodes compared to all other sites.

Fais *et al*. (1991) reported that Crohn's disease lamina propria in an *in vitro* culture spontaneously released more IFN-τ than lamina propria from healthy controls. IFN-τ increases expression of MHC class II antigens on these cells, an effect augmented as well by TNF (Adams and Hamilton, 1987). This leads to enhanced antigen presentation. IFN-τ also leads to a decrease in the number of suppressor T cells as well as the enhancement of the defective natural killing ability found in patients with IBD (Strickland *et al*., 1980). Both IL-2 and IFN-τ activate cytotoxic T cells and arm NK cells to become lymphokine activated killer cells leading to their effector functions (reviewed by Balkwill and Burke, 1989). High levels of these cytokines therefore may lead

to enhanced immune response and subsequent cytopathic effects.

Mucosal B cell function and cytokines

In in vitro experiments both rIL-2 and recombinant (rIFN-τ) increased immunoglobulin synthesis of pokeweed mitogen stimulated human lymphocyte in a dose dependent fashion. IL-2 enhanced the helper activity of mucosal T cells (James and Graeff, 1987).
The high levels of IL-2 and IFN-τ reported in IBD lead to increased expression of both surface IL-2R and MHC class II molecules on B lymphocytes. Both these effects are augmented by TNF. Furthermore the lamina propria has high numbers IL-5 producing cells as reported the in murine model (Taguchi et al., 1990). Interleukin-5 increases the expression of the 55kDa chain of the IL-2R (Loughnan et al., 1987). The overall effect is to upregulate the ability of B cells to present antigen and produce antibody in inflammatory bowel disease.

Cytokines and mucosal mast cells

Mast cells are a prominent feature of inflamed mucosa. There is mast cell hyperplasia in both forms of IBD. In vitro these mast cells displayed chemotaxis towards laminin, a major component of basement membranes

(Thompson et al., 1989). The chemotaxis increased when mast cells were stimulated with antigen. Intestinal epithelial antigens stimulated histamine secretion from intestinal mast cells in IBD but not in intestinal mast cells from non-IBD patients (Fox et al., 1987). TNF has recently been identified as a product of mast cells and basophils (Steffen et al., 1989).

The effect of cytokines on intestinal mucosa

Human IFN-τ and IL-4 enhance the expression of secretory component in HT 29 epithelial cell lines (Phillips et al., 1990). Campbell et al. (1988) reported that individually or in combination TNF or IFN-τ were capable of destroying isolated murine ß-islet cells in vitro.

The role played by IL-1 in the gastrointestinal tract is yet to be fully elucidated. IL-1 directly inhibited rabbit ileal mucosa ion transport in vitro by inhibiting sodium and chloride absorption (Chinosone, Simon and Smith, 1990). This feature and its proinflammatory effects suggests that high levels of IL-1 in vivo would lead to mucosal inflammation and diarrhoea. Indeed the serum level of IL-1 was raised in IBD patients compared to healthy controls (Ligumsky et al., 1990). However, whereas IL-1 was significantly raised in the early stages of experimental immune complex colitis induced in

rabbits, IL-1 itself inhibited both the acute inflammatory infiltrate and the subsequent necrosis (Cominelli et al., 1990). Furthermore in mice IL-1 was also found to be protective in radiation induced intestinal mucosal injury (Wu and Miyamoto, 1990). The effect of the increased levels of IL-1 found in inflammatory bowel disease could be to limit or repair the ongoing damage. In IBD, the beneficial effects of IL-1 may be limited by the presence of high levels of IL-1 antagonists such as IFN-τ (Donnelly et al., 1990), or IL-1 natural inhibitor. The co-factor for the production of the latter is granulocyte-monocyte colony stimulating factor which is also raised in IBD (Mazzei et al., 1990).

Tumour necrosis factor may also play a protective role as shown in leishmaniasis. Tumour necrosis factor promotes the generation of nitric oxide which is one of the effector mechanisms for intracellular killing (Liew, Li and Millot, 1990).

Conclusion

The initial role of these cytokines in experimental immune complex colitis is protective. There is however little doubt that some of these cytokine such as TNF do cause tissue injury as discussed in chapter 4B. Perhaps there is an optimum TNF response level for normal

function, so that very high or very low levels are deleterious.If infection is the trigger for IBD then IBD may be the result of a defect in downregulation of the immune response after an appropriate effective immune reaction. Persistence of the antigen may also provoke continued cytokine production. In either case the blocking of the inappropriately high cytokine activity would be beneficial to the host.

As this chapter shows cytokines play a prominent role in gastrointestinal immunology. All the work cited above has either been done in vitro, with animal models, or in sera. Any or all of these may not reflect accurately in vivo intestinal immune activity. In this thesis a novel approach was used to study mucosal T cell activity by measuring cytokine levels in whole gut lavage fluid. The levels of sIL-2R in the serum and whole gut lavage fluid of controls and IBD patients are discussed in chapter 12 and chapter 13 is a study of TNF levels in WGLF of controls and IBD patients. These factors were also studied in patients who were prescribed elemental diet.

CHAPTER FIVE:

ELEMENTAL DIET: HISTORY AND POSTULATED MECHANISMS OF ACTION IN IBD

Introduction

Elemental diets (ED) are chemically defined diets composed of glucose polymers and sucrose as a source of carbohydrates, essential and non-essential amino acids, minerals, lipids and trace elements (see Table 5:1 at the end of this chapter, p88). They are also sometimes referred to as defined formula diets, but this term includes diets containing polymeric peptides as a source of protein. Elemental diets provide complete nutritional support. There is no fibre or lactose in elemental diets. The chemical compositions of the three types of defined formula diets are shown in TABLE 5:2.

TABLE 5:2 Compositions of three types of defined formula diets.

Elemental	10% amino acids
	85% carbohydrate
	5% fat
Polymeric	15% polymeric peptides
	50% carbohydrates
	35% fat
Hydrolysed Formulae	10-15% oligopeptides
	60-80% polysaccharide/ oligosaccharides
	10-35% fat

The general uses of elemental diets

An elemental diet was developed for potential use in space travel as it has a low residue. In 1965 Winitz et al. tested the diet on volunteer prison inmates. As expected elemental diet provided complete nutrition without any adverse effects with the added advantage of a very low residue.

The first documented use of elemental diet in disease was by Stephen and Randall (1969). They used elemental diet in disease because they thought it was hypoallergenic, easily absorbed and would provide full nutrition. Stephen and Randall treated patients with a number of high catabolic states arising from different conditions. These included patients with short bowel syndrome, pancreatitis, biliary fistula, ulcerative colitis, duodenal Crohn's disease and oesophageal-tracheal-pleural-cutaneous fistula. They all benefited. The use of elemental diets in inflammatory bowel disease is discussed below.

Elemental diet in inflammatory bowel disease

History

Stephen and Randall (1969) used elemental diet (ED) in patients with inflammatory bowel disease (IBD) on the grounds that since this diet did not have any polypeptides or other antigens, it would be hypoallergenic to the patients. In addition it would provide nutrition which is readily absorbed without leaving any residue. The diet was found to be of benefit in both ulcerative colitis and Crohn's disease improving nutrition and decreasing disease activity.

Voitk _et al_. (1973) treated 13 patients for an average of 22 days with elemental diet. All but one showed weight gain. Occasionally indications for surgery resolved during elemental diet nutrition. They wondered whether this was the natural course of disease or a primary therapeutic effect of the diet. There was also a reduction in steroid-associated nitrogen losses in patients on ED and a positive nitrogen balance could be induced even in the presence of toxic reactions, without aggravating the colonic disease process.

Axelsson and Jarnum (1977) treated 34 patients (23UC, 11CD) with elemental diet. Thirty-one of these had had high dose prednisolone therapy 1 to 4 weeks prior to the diet with no or insufficient response. Fifteen of the 34

(44%) went into remission on a regime of elemental diet alone. A further 6 (18%) showed disease remission when steroids were added.

O'Morain, Segal and Levi (1980) studied 32 patients with acute Crohn's who were prescribed elemental diet alone for four weeks. Twenty-nine went into remission and they suggested that this remission was due to the fact that the diet did not include any antigenic molecules. They also suggested that the diet may act by altering bowel flora. This could be important as many workers have shown evidence for differences in intestinal bacterial flora between patients with IBD and healthy controls. Matthews et al. (1980) found that the sera from Crohn's disease had higher numbers positive agglutination results with Eubacterium and Peptostreptococcus than sera from healthy controls. They also reported that a complement fixation test against Chlamydia gave more positive reactions with sera from IBD patients than healthy controls. Various bacteria have been implicated as discussed in chapter 1A. It is therefore probable that a change in this bacterial balance may lead to beneficial effects for IBD patient.

Le Quintrec et al. (1987) showed that these diets not only induced clinical remission but in some cases allowed steroid withdrawal in patients with corticosteroid resistant and corticosteroid-dependent forms of Crohn's disease. The patients on the diets showed an increase in

haemoglobin and serum albumin.

Controlled trials of elemental diet

Comparison with standard steroid therapy

O'Morain, Segal and Levi (1984) conducted the first controlled randomised trial of the efficacy of elemental diets compared to standard steroid therapy. They randomised 21 acutely ill patients with exacerbations of Crohn's disease into two groups. Ten received 0.75mg/kg prednisolone and 11 received elemental diet for 28 days. The patients were assessed at 4 and 12 weeks. In both groups 80% went into remission by the fourth week. Clinical improvement started six to seven days before positive nitrogen balance was attained.

Saverymuttu, Hodgson and Chadwick (1985a) also compared prednisolone with a combination of elemental diet and non absorbable antibiotics in active Crohn's disease. Both the two types of treatment led to an improvement in Crohn's disease activity index, erythrocyte sedimentation rate, and reduction in their excretion of radioactive Indium labelled autologous leukocytes in the faeces.

Recently O'Keefe et al. (1989) randomised 6 patients with acute ileal Crohn's disease to either steroid therapy or an elemental diet. There was clinical improvement in all six patients with reductions in ESR, platelets, and an

increase in albumin. Both groups showed an increase in whole body protein turnover and the rates of incorporation of amino acids into albumin. The increase in the rate of protein metabolism induced by steroids was at the expense of body protein stores resulting in a net loss of 58g in 7 days. Both groups of patients showed reduced numbers of lymphocytes subsets, complement and circulating immune complexes. The steroids also suppressed *in vivo* IgG synthesis.

Okada *et al*. (1990) compared an exclusive elemental diet with prednisolone in the treatment of active Crohn's disease. After six weeks they found that the simple activity index, body weight, ESR, CRP and alpha-2 globulin and radiographic findings improved significantly in the patients on elemental diet. Even those patients who did not initially respond to steroids improved both in terms of their bowel lesions and clinically after being switched to elemental diet.

The multicentre European Co-operative Crohn's Disease Study III (Malchow *et al*., 1990) involving 95 patients arrived at different results. In this trial the efficacy of treatment of active Crohn's disease by means of a liquid defined diet was compared with treatment with a combination of 6-methyl-prednisolone and sulfasalazine. By the end of six weeks the drug treatment group showed a significant improvement of the Crohn's Disease Activity Index ($p<0.05$). Further analysis of the patients who

finished the study showed no difference between the two groups. Their final conclusion was that drug therapy was superior to liquid defined formula diet in Crohn's disease. It is important to emphasise here that this study used a protein hydrolysate-containing defined diet and not an elemental diet in the proper sense. They chose this form of diet because of its proven better absorption (Smith, Arteaga, and Heymsfield, 1982) and utilisation (Silk et al., 1980).

Elemental diet versus polymeric diets in IBD

The results of these trials comparing elemental and polymeric diets continue to be contradictory and therefore inconclusive.

Cosnes (1988) compared the nutritional efficacy of elemental and polymeric nutritive mixtures in Crohn's disease. After 28 days there was no significant differences in weight and anthropometric measurements between patients on exclusive enteral diet, polymeric or elemental. Transferrin and albumin, however improved much faster in the exclusive elemental group. This certainly implies some additional effect on inflammation.

Giaffer, North and Holdsworth (1990) conducted a controlled randomised trial comparing an elemental diet (16 patients) and a protein hydrolysate containing liquid preparation (14 patients) in patients with active Crohn's

disease. Twelve patients (75%) receiving elemental diet achieved clinical remission compared to only five (36%) remission among those receiving the hydrolysed diet ($p<0.03$). The fall in the CDAI in the group treated with elemental diet was statistically significant ($p<0.01$). Laboratory findings, haemoglobin, ESR, platelet count, plasma albumin and alpha-1 acid glycoprotein and anthropometric measurements, arm and skin fold thickness, showed no change in either group.

Two recent trials have shown different results. Park et al. (1991) randomised seven patients to either the polymeric or elemental diet for 28 days. They found no difference in the disease remission rate or indices of disease activity between the two groups. Raouf et al. (1991) also reported that there was no difference between the two diets and they suggested that the therapeutic effect could be a result of nutritional improvement other than a specific anti-inflammatory effect.

There are a few postulates as to the mechanism of action of elemental diets in IBD and these are discussed below.

Mechanisms of action of elemental diet

Non-immunological mechanisms

Bowel rest

Some practitioners believe that elemental diet works by giving adequate nutrition to the body without taxing its digestive and absorptive systems of inflamed gut. Experimental evidence, however, does not support this theory. Greenberg et al. (1988) randomised 51 patients with acute IBD to complete parenteral nutrition (17), partial parenteral nutrition (15) and 19 to enteral nutrition. They reported that total parenteral nutrition was no better than partial parenteral nutrition or defined formula diets in inducing disease remission. Bowel rest therefore was not a factor in inducing remission in IBD. They also suggested that parenteral nutrition may be disadvantageous in that it denies the epithelial tissue enteral glutamine which might be important for its metabolism, differentiation as well as for tissue repair.

Non-specific nutritional effect

The suggestion that improvement in disease activity when on elemental diets is a result of generalised improved

nutrition (Raouf et al., 1991; Park et al., 1991) is not wholly supported by experimental evidence. In the first place polymeric diets which are better absorbed (Horibe, 1987; Smith et al., 1982) than elemental diet are not more effective than elemental diets at inducing disease remission (Giaffer et al., 1990; Cosnes et al., 1988). In addition whenever elemental diets are effective, the benefit becomes apparent within 4-7 days before there are any nutritional changes in the recipients (Teahon et al., 1991; O'Morain et al., 1980; 1984; Giaffer, North and Holdsworth, 1990). These observations make a simple nutritional effect unlikely as a primary mechanism.

Other workers have proposed that elemental diets act by decreasing gut permeability (Teahon et al., 1991) and therefore intestinal protein losses (Logan et al., 1981). That gut permeability decreases with elemental diet nutrition is supported by the study by Sanderson et al. (1987) of fourteen children with Crohn's disease. All the children had abnormally raised lactulose/rhamnose permeability ratios which fell significantly after six weeks of elemental diet. This change coincided with marked clinical improvement as assessed by CDAI. However the change in permeability could be the result rather than the cause of improvement. This has been discussed in chapter 1 under permeability factors.

Effect of elemental diet on gut flora

The effect of elemental diets on gut flora has been studied with varied and inconclusive results.

Menge et al. (1985) found no differences in the small intestinal flora in rats between the group fed elemental diet for 60 days compared to a matched control group. In both groups there were similar numbers of aerobic and anaerobic colony forming bacteria per ml of jejunal juice.

Two studies, one involving human volunteers (Crowthen et al., 1973) and one an animal study (Kehoe, Harvey and Daly, 1986) found some difference.

Crowthen et al. (1973) showed that consumption of elemental diet altered both faecal flora and faecal steroid levels in three normal volunteers. Enterobacteria increased while enterococci and other lactic acid bacteria decreased in number. Numbers of Streptococci viridans were reduced in two of the three subjects. However the total number of organisms per gram of faeces remained at the control value of 10^{10}-10^{11}.

Kehoe, Harvey and Daly (1986) reported that when rats were given elemental diet in combination with methotrexate small bowel luminal cultures showed an increase in the numbers of E. coli and Pseudomonas species. Histology showed severe small bowel mucosal enteritis in comparison with rats on a regular diet. In

the absence of cytotoxic drugs there was a predominance of coliform bacteria and enterococci (Stanford et al., 1979).

No firm conclusions can be made from these studies regarding the effect of elemental diet on intestinal flora. A controlled study of a large number of human volunteers may resolve this question.

Immunological mechanisms

Inflammatory processes may be involved in the primary pathogenesis of IBD and there is evidence of immune upregulation in the established disease as discussed in chapters 1,2 and 4. The potential antigenic substances include resident and traveller bacterial flora and their constituents (chapter 1) as well as food antigens (chapter 2). The changes in antibody activity, in the complement system and cytokines (chapter 4) also adds further support to involvement of the immune system in IBD. Chapter 9 of this thesis addresses the question of food antibodies levels in IBD patients compared to healthy controls.One of the long standing theories is that since elemental diets do not contain any potentially antigenic material, they act as hypoallergenic sources of nutrition. Chapter 10 describes changes in antibody levels observed in patients on elemental diet.

The acute lesion in IBD

There is a large infiltration of the mucosa and submucosa with neutrophils in the acute lesion of Crohn's disease (Saverymuttu et al., 1985b) and ulcerative colitis (Saverymuttu et al., 1985c). This infiltration may be due to the presence of chemotactic factors such as the cytokines in the intestinal lumen (as discussed in chapter 4). There is need for a study of cytokine activity in IBD with respect to elemental diet therapy so as to delineate the role of the T cell arm of the immune system in disease response. The IL-2 system which is pivotal to all T cell immune activity and TNF which mainly secreted by activated macrophages can be studied as a measure of T cell and macrophage activity respectively. The changes in T cell and macrophage activity are part of the subject of this thesis and are discussed in chapters 12 and 13.

Summary and conclusion

Elemental diets can induce clinical remission in IBD, although it is not proven an immunological mechanism probably predominates. The determination of the mode of action of elemental diets in IBD is not without obstacles. In the first instance there is the difficulty of determining disease activity in IBD. Among the many

indices the most widely used has been CDAI for Crohn's disease. The problem with this is that the most subjective variables such as the severity of abdominal pain and general well being account for close to 39% of the index and the haematocrit is the only laboratory parameter (reviewed by Bartholomew and Shearman, 1989). A more reliable and simple way of measuring disease activity is the measurement of IgG levels in whole gut lavage fluid. The development of this method of measuring disease activity is described in chapter 7.

Whole gut lavage fluid was also used to study the changes in secretory immunity that occur when IBD patients are put on elemental diet. Serum provided material for the study of systemic immunity.

TABLE 5:1 CONSTITUENTS OF ELEMENTAL eo28

Composition	per 100g
Energy kJ	1568
kCal	370
Protein Equivalent (g)	10.0
Amino acids (g)	12.0
Fat (g)	6.64
of which	
saturates	17.1
monounsaturates	56.9
polyunsaturates	26.0
Carbohydrate (g)	7.2
Vitamins and trace elements	

CHAPTER SIX:

MATERIALS AND METHODS

SECTION 6A:

GENERAL METHODS

PATIENTS

The patients studied were those under routine medical care of the Physicians and Surgeons in the Gastroenterology Unit of the Western General Hospital.
The diagnosis of ulcerative colitis and Crohn's disease was based on clinical findings, radiology, endoscopy as well as histopathology.
Controls comprised of individuals who were referred to any of our consultants for further investigation of symptoms and in whom no abnormality was detected and healthy volunteers.

INFORMATION MANAGEMENT

Patient details were obtained by personal interview as well as by reviewing patient hospital records. The information was stored in data base files using a dbase III plus programme on an IBM compatible computer. The files contained fields such as name, date of birth, date of lavage, drugs, smoking habit and the results of clinical and experimental investigations.

STATISTICS

Minitab 7.2 programme was used for the statistical analysis. Most of the work involved correlations between

various measured parameters and ascertaining the magnitude and significance of differences in antibody levels or cytokine levels. The data was non-parametric and it is presented as medians and ranges. Spearman's correlation coefficient was used for correlations between any two variables and regression analysis performed to determine the significance of the correlations. The Mann-Whitney U test was done for comparisons between groups and Wilcoxon rank sum test for paired data. In all the tests $p < 0.05$ was accepted as significant and $p < 0.01$ as highly significant.

ASSESSMENT OF DISEASE ACTIVITY AND DISTRIBUTION

Disease activity was based on a number of criteria which included clinical assessment, Crohn's disease activity index, Powell-Tuck index for ulcerative colitis and total IgG concentration in whole gut lavage fluid. Disease distribution was based on radiological and endoscopic appearances and confirmed by histology.

COLLECTION OF SPECIMENS

COLLECTION OF SERUM

Venous blood was collected from the ante-cubital veins of the forearm using 21mm sterile disposable needles and

disposable syringes of varying capacity (20-60ml) (Becton-Dickinson, Dublin). Blood was drawn from all the patients and controls into plain tubes, stored at room temperature till clotted and then centrifuged at 1500g. The serum was aspirated and stored at -70^{o}C till assay for food antibodies. Blood samples for total plasma immunoglobulins were collected in lithium heparin treated containers and delivered to the Western general hospital clinical chemistry laboratories for assay.

COLLECTION OF SALIVA

Parotid salivary flow was stimulated with four 0.5 ml aliquots of 5% citric acid sublingually over five minutes, and saliva was collected via a Carlsson-Crittenden cup placed over the parotid duct using gentle aspiration to maintain both position and suction. Only stimulated saliva was collected.

COLLECTION OF WHOLE GUT LAVAGE FLUID

After an overnight fast the patients or control subjects were encouraged to drink 200ml of a polyethylene glycol (PEG) based electrolyte lavage solution, Golytely (see appendix of this section) every 10 minutes till the material passed from the rectum became liquid, clear and free of faecal mater. Clear liquid material was usually

obtained within 4 hours after about 2-2.5 litres of golytely had been ingested.

Approximately 100ml of this clear fluid was collected, filtered twice and treated with protease inhibitors (see appendix for details). The specimens were stored at -70^{o}C till assay.

COLLECTION AND ISOLATION OF HUMAN LYMPHOCYTES FROM PERIPHERAL VENOUS BLOOD

Ten ml of blood was collected by venepuncture into a heparinised bottle. The blood was mixed with an equal volume of RPMI 1640 in a Universal container. The mixture (7.5ml) was gently layered on top of 2.5 ml ficcoll-paque in a conical centrifuge tube. The tubes were spun at 400g for 30 minutes in an MSE bench centrifuge. The white cells formed a layer at the interface between the ficoll-paque below and plasma above. The white cell layer was then aspirated into a sterilin tube and an equal volume of RPMI 1640 with 5% feotal calf serum added. After mixing well the cells were washed twice by centrifuging at a high speed close to 3000rpm for 2 to 3 minutes. The cells were finally resuspended in the medium at concentrations compatible with the planned experiment. The yield was between 1-1.7 million cells per millilitre of blood processed with a viability of 93-98% by trypan blue exclusion.

MEASUREMENT OF TOTAL IMMUNOGLOBULINS

TOTAL IMMUNOGLOBULINS IN SERUM

Total immunoglobulins of the IgG, IgA, and IgM class in sera were assayed by the Western General Hospital clinical chemistry department by an immunoturbidimetric method as part of a routine hospital service. Total immunoglobulins were reported in IU/ml. A concentration of 100IU/ml is equal to 1.69g per litre for IgA, 8.7g per litre for IgG and 0.87g per litre for IgM. Plasma total protein and albumin assays were also performed as part of a routine hospital service.

ELISA METHOD FOR THE MEASUREMENT OF TOTAL IMMUNOGLOBULINS IN SALIVA AND WHOLE GUT LAVAGE FLUID

Reagents

Human colostral IgA (Sigma, UK)
Goat anti-human IgA, IgG, IgM (NorthEast Biomedicals, UK)
Alkaline phosphatase conjugated Goat anti-human IgA,IgG,IgM (NorthEast Biomedicals, UK)
Human serum standard (SPS-01) (Sheffield Protein Reference Unit)
Paranitrophenylphosphate (PNPP) tablets or powder (Sigma, UK)

Principle

Test samples from patients and controls were compared to a standard curve constructed by serial two-fold dilutions of the human serum standard. For example for IgG and IgM assays doubling dilutions were made ranging from 1000 - 15.625ng/ml of a human serum standard. Serial dilutions of test samples starting with different concentrations depending on the test material were done (see below). A standard curve was then constructed by plotting the log of the concentration of the standards against the corresponding optical density (Graph 6A:1 in appendix of this section). Only when the optical density readings of at least two of the test sample dilutions fell within the range of the standard was the assay considered successful. The optical density of these two dilutions was then used to extrapolate the immunoglobulin concentration. The immunoglobulin concentration was taken as the mean of these dilutions. Human colostral IgA was used as the standard for IgA assays.

Procedure

Assays were performed in 96 well micro-titre plates (M129A, Dynatech). All reactants were added in volumes of 0.125ml per well and all washes were done three times using saline with 0.05% Tween-20. The details of the

composition of the solutions used are in the appendix of this chapter. The wells were coated with 1/5000 dilution of the goat anti-human class specific antibody in bicarbonate coating buffer (pH 9.6) incubated overnight and washed. ELISA diluent was then added and left for 30 min so as to block any remaining binding sites. After decanting, serial twofold dilutions of standard and samples (initial dilution 1/100 for IgA and 1/25 for IgG and IgM) were added to the coated wells. Plates were incubated overnight at 4^{o}C and washed. Goat class specific anti-human Ig alkaline phosphatase conjugate diluted 1/5000 in ELISA diluent was added and the plates were incubated for three hours at 22^{o}C. After washing, 1mg/ml PNPP in diethanolamine (DEA) buffer, pH 9.8, was added. Plates were read at optical density of 405nm with a 630nm reference filter in an MR5000 microELISA reader (Dynatech, UK). The immunoglobulin concentration of any given sample was determined as described above with adjustments for the dilution.

MEASUREMENT OF SECRETORY IMMUNOGLOBULIN A IN WHOLE GUT LAVAGE FLUID

Reagents and procedures were the same as for total immunoglobulins except for the coating antibody which was a mouse monoclonal anti-human secretory component (Sigma) diluted 1/2000. The assay was done in parallel with total IgA assays on the same plates.

ELISA METHOD FOR THE MEASUREMENT OF IgG, IgM, AND IgA ANTIBODIES TO OVALBUMIN, GLIADIN AND BETA-LACTOGLOBULIN IN SALIVA, SERA AND WHOLE GUT LAVAGE FLUID

Antigens

Gliadin (GLI; gift from Dr H. Weiser, DFA für Lebensmittelchemie, Garching, Germany).
Ovalbumin, five times crystallized (OVA; Sigma, UK)
Beta-lactoglobulin (Sigma, UK)

Standard

Serum from a patient with untreated coeliac disease who had high titres of antibody against most antigens was used as reference standard. The standard and test specimens were studied at suitable dilutions varying for the different assays (see below). The plates were read when the optical density of the standard reached 1.0 and the results expressed as percentage of the standard. These results therefore are non-parametric and are not directly proportional to the antigen binding capacity of the samples.

Procedure

Immulon 2 plates (M129B, Dynatech) were found superior to M129A plates used for total antibody assays as they bound antigen more avidly.

Wells were coated with antigen (GLI, BLG or OVA) at a concentration of 5ug/ml in bicarbonate coating buffer. The sample dilutions were as follows; for serum IgA and IgM antibody 1/100 and for serum IgG antibody 1/200; and 1/2 for all saliva and WGLF antibody assays. Reference standards and samples were added to the coated wells in duplicate. The dilutions for the reference standard were the same as for serum except for IgM antibodies where the standard was used at 1/10. Adsorption assays confirmed the specificity of these antibodies and the within plate coefficient of variation was 7.3% and the between plate optical density variation was 11.1%.

Interpretation of results of food antibody assay

Food antibodies were measured against a serum sample with known high antibody titres to most food antigens.

In serum, values above 10 were taken as being positive and values for IgA above 20, IgG above 40 and IgM above 60 were taken as showing high levels.

In whole gut lavage fluid (WGLF) and saliva any value above 10 was considered as high.

MEASUREMENT OF ALPHA-1 ACID GLYCOPROTEIN

Principle

When human α-1 acid glycoprotein (α-1AP) reacts with specific antibody in the presence of polyethylene glycol (PEG), precipitating immune complexes form. If the antibody is present in large excess, these precipitates produce a turbidity which is related to the concentration of α-1 acid glycoprotein in the sample. The turbidity is photometrically measured at the wavelength of 340nm. Absorbance readings obtained by assaying calibration standards are used to generate a standard curve, from which the concentration of α-1 acid glycoprotein in the sample is derived (Lubega and Jeffrey-Davies, 1990).

Reagents

All reagents are stored at $4^{o}C$.

PEG Reagent: 40g/litre polyethylene glycol 6000 (BDH-biochemical grade) in tris buffer (0.05M, pH 7.0) containing 2ml/litre Tween 20 and 1g/litre sodium azide.

Antibody Reagent: Goat anti-human α-1 acid glycoprotein (PRU - Sheffield UK) diluted 1/50 with the PEG reagent on the day of assay.

Diluent: A litre of diluent comprises 9g sodium chloride, 5g gelatin, and 1g sodium azide.

Calibration Standards: The following dilutions of the human calibration serum SPS-01 (PRU - Sheffield) were used for producing the standard curve: 0.18ug/ml, 0.36ug/ml, 0.73ug/ml, 1.46ug/ml, 3.65ug/ml and 7.3ug/ml.

Procedure

All standards and samples were tested in duplicate. The antibody reagent (950ul) was added to 50ul of one of the paired samples in 3 millilitres polystyrene tubes. To the other sample of the pair 950ul of PEG reagent without the antibody was added so that each sample had its own individual blank control. The blanks and tests were read after 15-20 minutes at 340nm using the spectrophotometer. A standard curve was constructed (see Graph 6A:2 in the appendix to this section) and the test sample and blank results extrapolated from this curve. The blank value is subtracted from the test sample reading and this is taken as the O.D. of the sample. Only the linear part of the curve was used. Samples that were above this level were further diluted and reassayed.

ELISA METHOD FOR THE MEASUREMENT OF INTERLEUKIN-2 RECEPTOR IN SERUM AND WHOLE GUT LAVAGE FLUID

Principle

The polystyrene microtitre wells are precoated with murine anti IL-2R monoclonal antibody. Standards or samples are introduced to the wells followed immediately by the addition of an enzyme conjugated anti-IL-2R monoclonal antibody. The two monoclonal antibodies specific for different epitopes on the released p55 component of IL-2R.

The soluble IL-2R present in the standards or samples binds to the coating antibody while the conjugated antibody binds to a second distinct epitope completing the sandwich. Unreacted components are removed by washing. A chromogen is then added to the wells forming a coloured end product that is proportional to the amount of soluble IL-2R in the sample. A stop solution (see below) is added to stop the reaction and absorbance read at 490nm. The concentration of the samples are determined from the standard curve constructed using the standards (see Graph 6A:3 in appendix).

Reagents

Ninety-six well microtitre plate coated with an murine anti- human IL-2R antibody.

Horseradish peroxide (HRP) conjugated murine monoclonal antibody to human IL-2R (T-Cell Science, Cambridge USA).

Specimen Diluent: Made up of Normal saline and 10% equine serum proteins and 0.01% thimerosal.

IL-2R standards: These are recombinant human IL-2R in a buffered solution with bovine serum albumin in doubling dilutions from 3200U/ml to 50ul/ml and 0U/ml.

Two control specimens: One for the low concentration and one for the high concentration.

6. O-Phenylenediamine Chromogen tablets (Sigma. UK.).

7. Substrate Diluent: A buffered solution containing 5.3mM/L urea peroxide and thimerosal.

8. Phosphate buffered saline (PBS pH = 7.2)

9. Stop solution: 2N H_2SO_4

Procedure

The blank wells were left empty and 50ul of standard, high and low controls and samples were pipetted into the antibody coated wells. HRP conjugated anti-IL-2R antibody (100ul) was added to all the wells except the blanks. All assays were done in duplicate. The plates were gently agitated by tapping, covered and incubated at room

temperature (24 ± 2°C) for three hours on a rotator set at 150 (± 10) rpm.

After incubation the plates were uncovered and the solution aspirated from all the wells. The wells were then washed 3 times and O-Phenylenediamine in substrate diluent was added. This was allowed to incubate for 30 minutes at room temperature before the addition of stop solution into all the wells.

Absorbance of wells was read at 490nm and if there were any values above the range at this wave length, the absorbance was read again at 450nm.

A standard curve was constructed using the means of each pair of standards (see Graph 6A:3 in appendix). The concentrations of IL-2R in the samples were extrapolated from the standard curves. In the pilot studies a few spiking experiments were performed, it is a costly assay. These showed that the results were reproducible though there was a loss of some soluble IL-2R of less than 10% (see Table 6A:3 in appendix).

ELISA METHOD FOR THE MEASUREMENT OF TUMOUR NECROSIS FACTOR ALPHA IN WHOLE GUT LAVAGE FLUID

The assay used for this was an enzyme amplified sensitivity immunoassay. It is based on the oligoclonal system in which several monoclonal antibodies (Mabs) directed against distinct epitopes of TNF-α are used. The

use of several distinct Mabs avoids hyperspecificity and allows high sensitive assays with wider range and short incubation time.

Reagents

The reagents were obtained from Medgenix (Milton Keynes, UK.) and comprised of the following.
Ninety-six well anti-TNF coated microtitre plates.
The following concentrations were used for constructing the standard curve 1500 pg/ml, 500pg/ml, 150pg/ml, 50pg/ml, 15pg/ml and 0pg/ml in human serum and merthiolate.
Incubation buffer: Tris-maleate buffer with 10% Bovine serum albumin
Anti-TNF-α-HRP conjugate in incubation buffer (Conjugate solution).
Tween-20
Chromogen Tetramethlybenzidine (TMB)
H_2O_2 in acetate/citrate buffer (substrate buffer).
1.8N H_2SO_4 stopping reagent.
Washing solution 0.05% Tween 20 in distilled water.

Standards and controls were reconstituted in distilled water. Substrate solution was made by pipetting 0.2ml of the chromogen TMB into 21ml of the substrate buffer. A 1% conjugate buffer was made by dissolving the conjugate

solution (Anti-TNF-α-HRP) in the conjugate buffer (TMB buffer).

Procedure

Standards, controls or samples (200ul) were dispensed into the wells of a microtitre plate followed by 50ul of the incubation buffer. The plates were incubated at room temperature on a horizontal shaker set at 700 (± 100) RPM for two hours.

The wells were then washed three times with 400ul of washing solution. Incubation buffer (100ul) was dispensed into all the wells followed by 50ul of anti-TNF-α-HRP conjugate. The plates were incubated again at room temperature on a horizontal shaker as above.

After a further three washes 200ul of the freshly prepared substrate chromogen solution was dispensed into each well. The plates were incubated for 30 minutes in a dark room with continuous shaking. The reaction was then stopped with the addition of 50ul of the 1.8N H_2SO_4.

The absorbance was read within the hour and the standard curve plotted (see Graph 6A:4 in appendix). The concentrations of TNF-α in the samples are extrapolated from the curve. Spiking experiments showed that most of the TNF could be accurately measured in whole gut lavage fluid (see Table 6A:4 in appendix).

APPENDIX FOR CHAPTER 6A

APPENDIX FOR CHAPTER 6A

GUT LAVAGE PROCESSING

REAGENTS

0.1g of soya bean trypsin inhibitor (SBTI; Sigma. UK)in 100ml PBS (pH 7.2)

11g 0.3M EDTA (pH 8.0) in 100ml of distilled water.

0.1M Phenylmethylsulphonylfluoride (PMSF) 1.74g PMSF in 100ml of 95% alcohol.

2% sodium azide in distilled water.

New born calf serum (NBCS)

Procedure

Collect 40ml of clear gut lavage fluid in two Universal Containers.

Filter 15-20 ml of lavage into a test tube.

Transfer 10 ml into a Universal container.

Add the following, mixing after each addition:

1 ml SBTI; 0.56 ml of 0.3M NaEDTA; 0.24 ml PMSF; 0.12 ml sodium azide.

Leave for two minutes

Add 0.6 ml NBCS and Mix

Dispense into 6 x 1.5ml aliquots into freezer tubes.

Store tubes at -70^{o}C.

SOLUTIONS

WHOLE GUT LAVAGE FLUID (WGLF)

Polyethylene glycol (GoLYTELY) (Seward Medical Ltd. London U.K)

236g polyethlene glycol 4000 BP

22.74g sodium sulphate BP

6.74g sodium bicarbonate BP

5.86g sodium chloride BP

2.97g potassium chloride BP

This mixture is reconstituted with water to a volume of 4 litres. The final solution contains;

125mmol/L sodium, 10mmol/L potassium, 40mmol/L sulphate, 20 mmol/L bicarbonate, 35mmol/L chloride, and 17.6 mmol/L polyethylene glycol 4000.

ELISA DILUENT

Physiological saline with 10% bovine serum albumin (BSA; SAPU, UK) and 0.05% Tween-20 (Sigma, UK)

ELISA WASH FLUID

Physiological Saline with 0.05% Tween-20 (Sigma, UK)

BUFFERS

10% DIETHANOLAMINE BUFFER

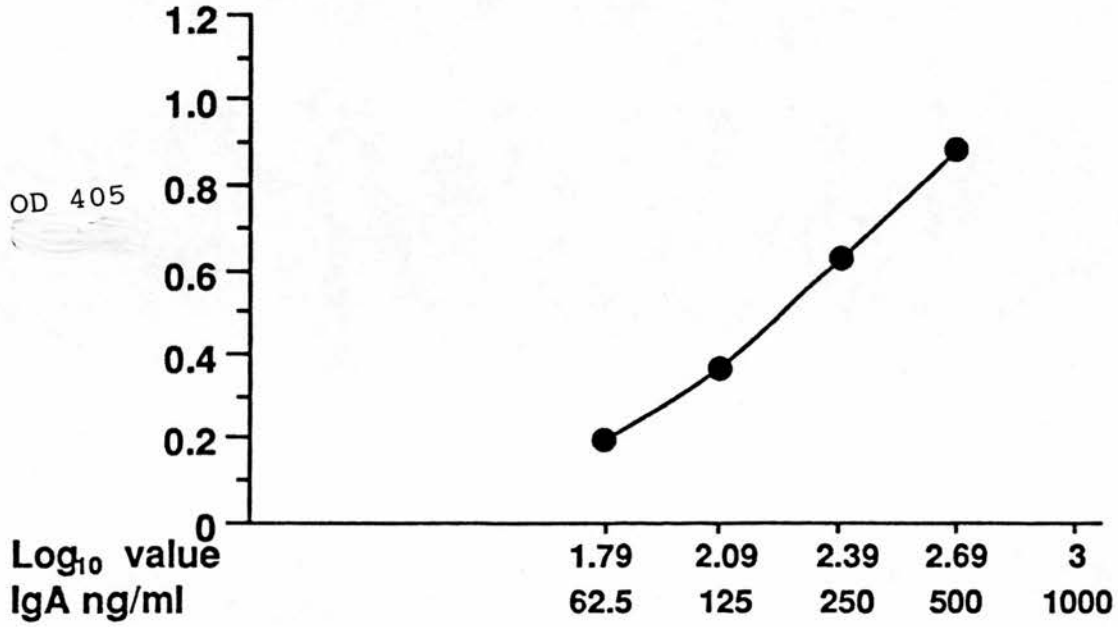
100mls Diethanolamine (DEA; BDH Ltd, UK) this is solid at room temperature, 800mls distilled water, 0.1015g $MgCl_2.H_2o$ (BDH Ltd, UK),0.2g Sodium azide (BDH Ltd, UK). Used at pH 8.6.

COATING BUFFER

Reconstituted by adding 900ml of distilled water to a 100ml Sodium carbonate/ bicarbonate coating buffer concentrate at PH=9.6 (NorthEast Biomedicals, UK).

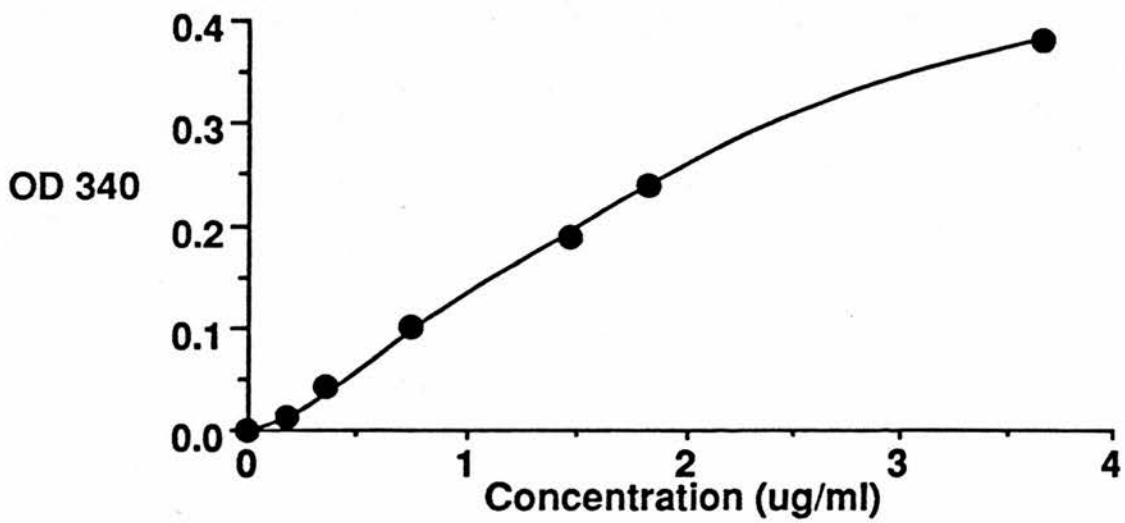
Graph 6A:1

STANDARD CURVE FOR THE ASSAY OF TOTAL IMMUNOGLOBULINS IN WGLF AND SALIVA



Graph 6A:2

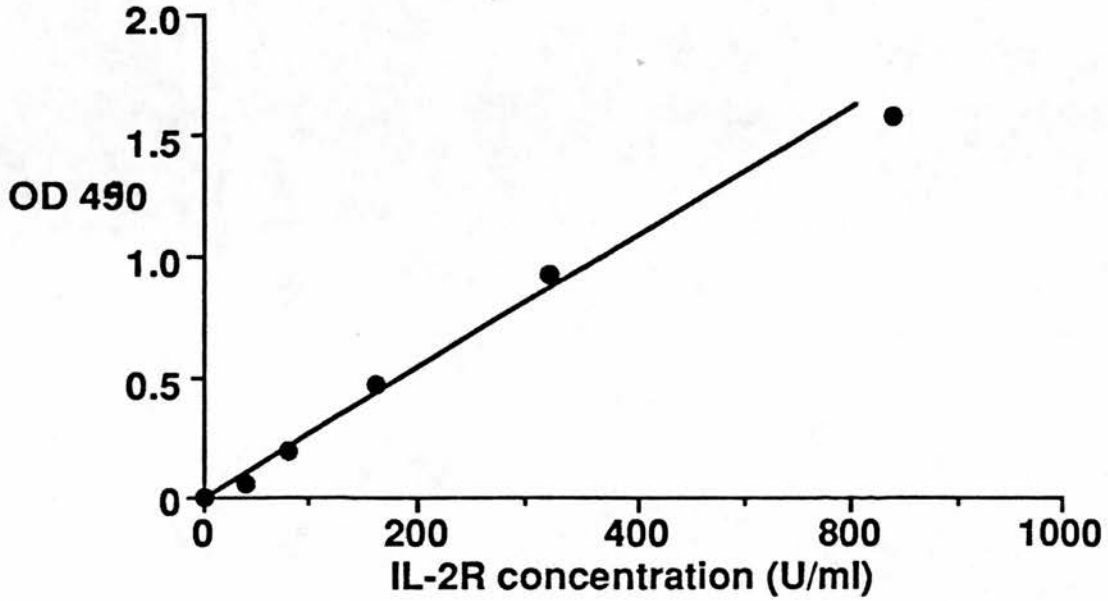
STANDARD CURVE FOR THE ASSAY α-1 ACID GLYCOPROTEIN



Standard curve used for extrapolation of total immunoglobulins and secretory IgA in gut lavage fluid and parotid saliva (top) and α -acid glycoprotein (bottom).

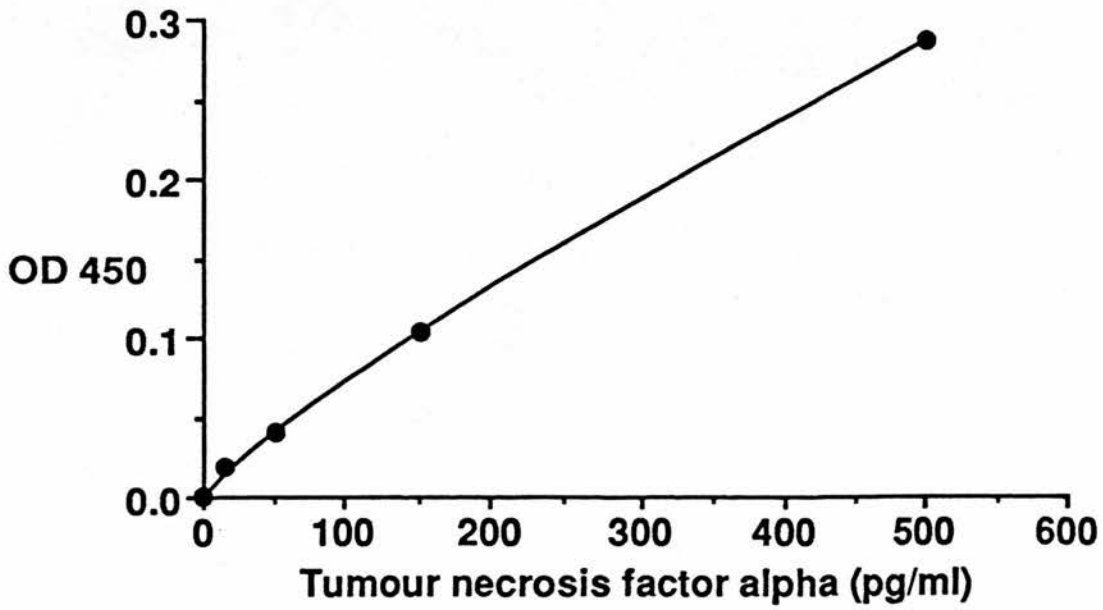
Graph 6A:3

STANDARD CURVE FOR THE ASSAY OF CELL FREE INTERLEUKIN 2 RECEPTOR



Graph 6A:4

STANDARD CURVE FOR THE ASSAY OF TNF IN WGLF



Standard curve used for extrapolation of soluble IL-2R levels (top) and TNF-α levels (bottom). A standard curve is constructed wih every assay.

TABLE 6A:3 WGLF sIL-2R SPIKING EXPERIMENTS.The table shows mean values of the levels of sIL-2R in WGLF before (BEFORE) addition of recombinant sIL-2R, the expected values after addition (EXP), actual values obtained (ACTUAL) and percentage difference from the expected value (%Dif).

sIL-2R LEVELS WGLF(U/ml)

NAME	BEFORE	EXP	ACTUAL	%DIF
SJ	34	354	360	1.8%
RC	10	330	320	3.0%
SB	0.00	320	310	3.1%

All the specimens were assayed on the same plate and in duplicate.

EXP = The expected sIL-2R level in U/ml when 10ul of a 1600U/ml standard has been added (spiking) to 40ul of the lavage fluid

BEFORE = The sIL-2R levels in the plain lavage specimens

ACTUAL = The actual levels obtained in the specimen after spiking.

%DIF = The difference from the expected level divided by the expected value and multiplied by 100.

TABLE 6A:4 WGLF TNF SPIKING EXPERIMENTS. The table shows mean values of the levels of TNF in WGLF before (BEFORE) addition of recombinant TNF, the expected values after addition (EXP), actual values obtained (ACTUAL) and percentage difference from the expected value (%Dif).

TNF LEVELS WGLF(pg/ml)

NAME	BEFORE	EXP	ACTUAL	%Dif
RC	0.00	100	89.5	10%
CC	1.9	102	97	5%
MA	30	130	125	4%

All the specimens were assayed on the same plate and in duplicate.

EXP = The expected TNF level in pg/ml when 50ul of a 500pg/ml standard has been added (spiking) to 200ul of the lavage fluid

BEFORE = The TNF levels in the plain lavage specimens

ACTUAL = The actual levels obtained in the specimen after spiking.

%DIF = The difference from the expected level divided by the expected value and multiplied by 100.

SECTION 6B:

ADAPTATION OF ELISPOT

SECTION 6B

STANDARDISATION OF THE ENZYME LINKED IMMUNOSPOT (ELISPOT) ASSAY

A series of experiments was set up to standardise the ELISPOT assay (see chapter 2C) using mouse splenocytes prior to using it to study antibody producing cells in human peripheral blood against three common dietary antigens; ß-lactoglobulin (BLG), ovalbumin (OVA) and gliadin (GLI). The first experiments were done with murine splenocytes to determine the following criteria.

1. The optimum coating surface.
2. Optimum coating antigen concentration
3. The optimum concentrations of coating antibody and coating antigen.
4. The optimum concentration of conjugate.
5. Whether modifications to the substrate would improve the results.
6. The best time to read the plates.

IMMUNISATION TECHNIQUE

Mice (BDF_1) (obtained from the animal unit, Western General Hospital) were immunised intraperitoneally (i.p.) with 100ug of one of the three dietary antigens suspended in 50ul complete Freund's adjuvant (CFA) (H37 Ra, Difco,

UK). The animals also received a booster injection of 100ug of antigen in incomplete Freund's adjuvant seven days after primary immunisation.

PREPARATION OF SPLENOCYTES

Immediately after sacrifice of mice by cervical dislocation the spleens were removed and dissected free from surrounding tissue. The spleens were then placed on a petri dish containing ice cold RPMI 1640 medium (Flow Labs, Irvine, UK) with 10% feotal calf serum (FCS; Gibco, UK)

The spleens were cut into smaller pieces and the segments passed through a wide pore sterilised gauze to remove cell clumps and connective tissue. Clumps were allowed to settle for 10 minutes on ice and the supernatant suspension was transferred to clean tubes. After centrifugation for 10 minutes at 400g, the pelleted cells were gently resuspended and exposed for 10 seconds to 4.5ml of distilled water to cause red blood lysis and quickly rescued with 0.5ml of 10X PBS. The ensuing cell suspensions were then washed three times in RPMI with 10% FCS at 400g in an MSE bench centrifuge.

The cells were resuspended in 5ml of RPMI with 10% FCS. Finally they were counted in an improved Neubauer haemocytometer using white cell diluting fluid.

The ability to exclude trypan blue was used to determine

cell viability. The viability was generally between 90-95%. The cells were finally resuspended in complete medium (see solutions) to cell concentrations found appropriate for the individual assays.

Procedure (illustrated schematically in Figure 6B:1)

Antigen or antibody in coating buffer (pH 9.6) was added to the wells and incubated overnight at $4^{o}C$ in a 'moist chamber'. The plates were then washed three times with normal saline (without tween). The final cell suspensions are made up in complete medium (see appendix) and added to the wells in appropriate cell densities. The plates were then incubated at $37^{o}C$ in 5-10% CO_2 humidified chamber for 4hrs. They were then washed with ELISA wash (0.05% tween 20 in normal saline) and the appropriate conjugate added followed by incubation at room temperature for 3hrs. Unreacted conjugate was washed three times with ELISA wash and a chromogen (5-bromo-chloro-indolyl-phosphate) added. After about 10-20 minutes blue spots became visible and these were counted either directly or under a low power dissecting microscope (see Figure 6B:2 and Figure 6B:3).

Results

Initially twenty-five well tissue culture grade plates (Repli-dishes; Sterilin, UK) were used as the coating surface but later 96-well microtitre plates were found to do as well. The M129B yielded more spots than the M129A under the same conditions (Table 6B:1 in appendix)

Experiments with different concentrations showed that the best concentration was 1mg/ml for all the three antigens as it gave the most spots with the least number of false positives (see Table 6B:2 in appendix).

Checkerboard experiments for immunoglobulin secreting splenocytes were performed. The wells were coated with varying concentrations of antibody along the rows in triplicate. After cell incubation conjugate, was added in varying concentration along the columns and the counts plotted. The best results were obtained with a conjugate dilution of 1:1000 for IgA (Graph 6B:1) and IgM (Graph 6B:2) while for IgG the best results were obtained with 1:2500 (Graph 6B:3).

The murine splenocytes were incubated at 37^{o}C for 3hours in 90-100% humid chamber with 5% carbon dioxide. Human peripheral blood antibody secreting cells requires 5 hours or overnight incubation for the best results.

The substrate 5-bromo-4-chloro-3-indolyl phosphate (see solutions) was initially mixed with agar gel to give a 0.6% solution which provided a solid base for the spots.

The use of gel matrix for the substrate gave a few problems. It gave artifacts which had to be distinguished from the true spots usually by the use of a low power magnifying microscope. The number of spots also tended to vary with time as illustrated in the appendix (Graph 6B:4 and Graph 6B:5). The possible reasons for this include the following; the bigger spots tend to become more diffuse as they spread in the gel this makes them less distinct, the smaller spots grow with time till they become visible and therefore countable. Persistently adherent B cell membranes may sometimes be detected by the developing agent. The latter artifacts take more time to develop than secreted antibody (Hutchings et al., 1989). Washing and using EDTA in the wash after cell incubation can reduce the last phenomenon. Therefore at a given time it will be the sum total of these effects that determined the count. Omission of agar in the substrate solution gave better results in that they were more consistent and had minimal numbers of artifacts (Plate B in the above Graphs 6B:4 and 6B:5).

Section 6C is an animal experiment comparing the sensitivity of ELISPOT and ordinary ELISA.

APPENDIX FOR SECTION 6B

APPENDIX FOR SECTION 6B

COMPLETE RPMI 1640 MEDIUM

RPMI 1640 (Flow Labs, Irvine, UK)	-	445 ml
Fetal Calf Serum (Gibco; UK)	-	50 ml
Hepes	-	5ml
Glutamine	-	0.5ml
Gentamycin	-	0.05ml

This makes up a solution of 10% FCS, 0.1% glutamine, 1% Hepes and 0.01% Gentamycin in 500ml of the complete medium.

BCIP STOCK SOLUTION

2-Amino-2-methyl-1-propranol solution (Sigma, UK)

Triton X-405 (Sigma, UK)

5-Bromo-4-chloro-3-indolyl phosphate (5-BCIP; Sigma, UK)

To prepare one litre of stock 2-amino-2-methyl-1-propranol buffer,

Warm neat 2-amino-2-methyl-1-propranol solution to 25-30^{0}C. It is solid at room temperature.

Dissolve the following in 500ml distilled water:

150mg $MgCl_2.6H_2O$; (BDH Ltd, UK)

100ul Triton X-405 and 1.0 g sodium azide. (BDH Ltd, U.K.)

To this solution, add 95.8ml (1.0M) amino-methyl-

propranol solution with stirring and leave for about 1hr. (amino-methyl-propranol is slow to dissolve). Solution may still be cloudy after this time.
Add distilled water to approximately 900ml and adjust pH to 10.25 with concentrated HCl (BDH Ltd, UK).
Leave overnight at 20^0C, re-adjust pH to 10.25 with HCl, and add water to 1 litre. Filter and store.
Stock buffer will store at 4^0C for at least 3-4 months but may require refiltering with age.

The 5-BCIP substrate solution is prepared fresh at a final concentration of 1.0mg/ml.
Take desired amount of 5-BCIP and dissolve in a small volume of stock AMP buffer, then make up to correct volume. Filter the solution through a 0.22 or 0.45um filter. This step is to remove any insoluble material, as particles in the substrate may give false spots. The resulting solution is a clear, lightish brown liquid (colour dependent on the purity of the 5-BCIP - the Sigma product is almost colourless).

TABLE 6B:1 A COMPARISON BETWEEN M129A AND M129B ELISA PLATES FOR THE ELISPOT ASSAY FOR ANTIBODY-SECRETING CELLS. The table shows the number of spot forming cells secreting IgM or IgG antibody specific for each of the three dietary antigens.

Spot Forming Cells/10^6 splenocytes

	Type of Plate			
	M129B		M129A	
	IgG	IgM	IgG	IgM
OVA	440	1100	200	30
BLG	830	95	400	10
GLI	100	75	70	36

M129B gave higher numbers than M129A plates for all antigens tested. This could be because M129B plates bind antigen more avidly than M129A (Czerkinsky et al., 1983).

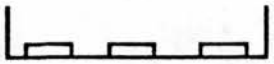
TABLE 6B:2 EFFECT OF ANTIGEN CONCENTRATION

	IgG spots/10^6 splenocytes		
Antigen (mg/ml)	GLI	OVA	BLG
2	35	440	800
1	38	436	1164
0.5	28	396	858
0.25	20	180	52

Optimum results, both in terms of clarity and numbers were obtained with the coating concentration of 1mg/ml of antigen .

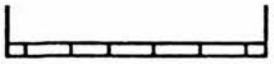
Figure 6B:1

THE ADAPTATION OF THE ELISPOT ASSAY FOR ANTIBODY SECRETING CELLS AGAINST ANY ANTIGEN (ie GLIADIN)

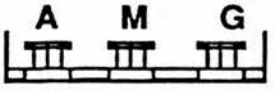
1) 

Coat with antigen

Wash x 3 (No azide, no tween)

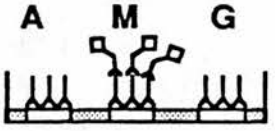
2) 

Block with culture medium containing 10% FCS or NCS

3) 

Add the cells and incubate

Wash x 3 with PBS tween.

4) 

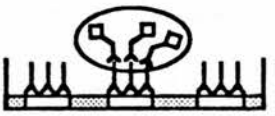
Add conjugate and leave at room temperature 3-5 hours

Wash x 3 with PBS tween

5) 

Add substrate

Decant substrate

6) 

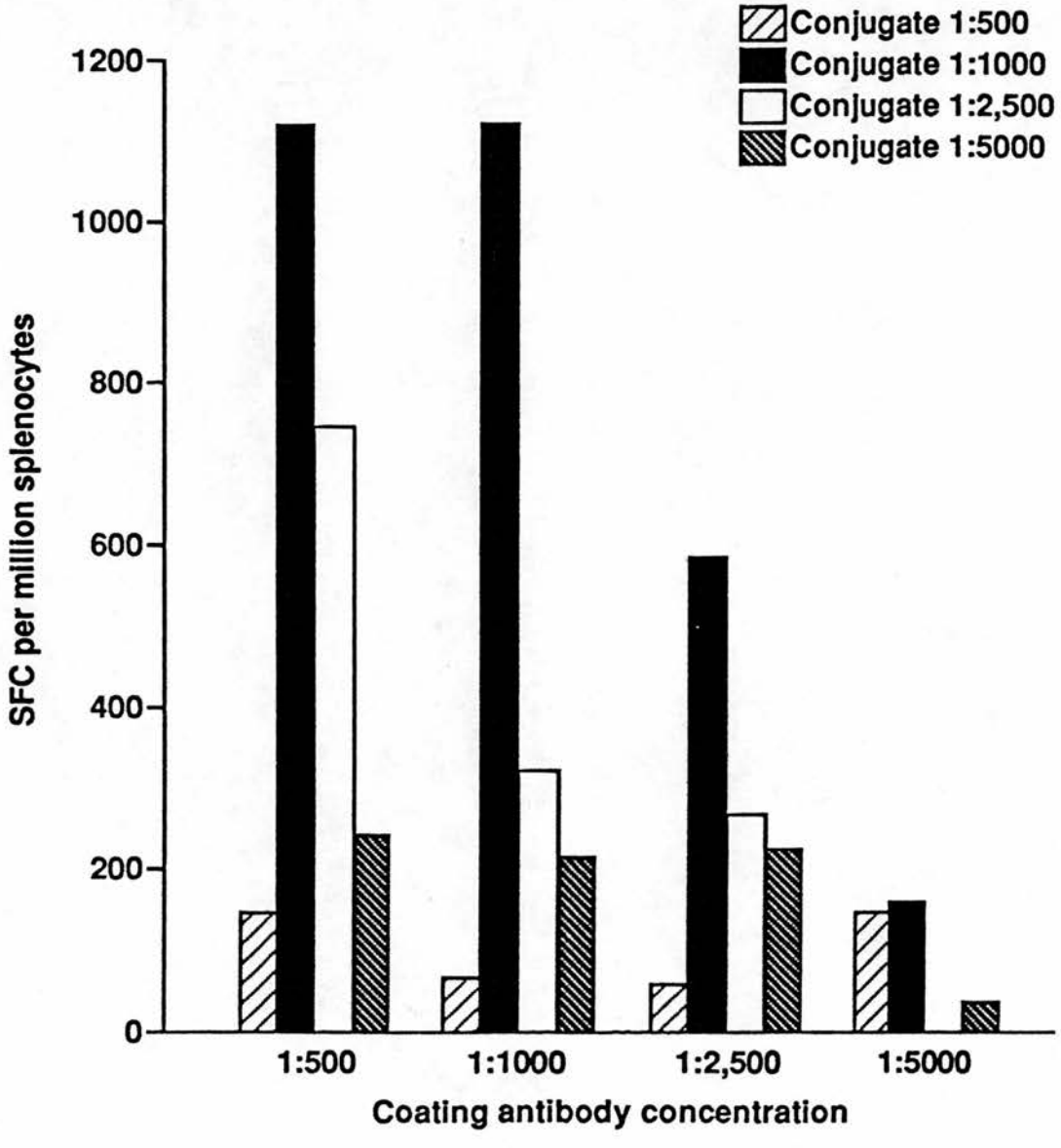
Wash and count spots.
Count of spots=counts of anti-(Gli) IgM producing cells

Figure 6B:2 Showing blue spots in one of the wells in a twenty-five well tissue culture grade plate. The blue cross was made outside the well as a marker for the well

Figure 6B:3 showing blue spots in the wells of a 96-well ordinary ELISA plate.

Graph 6B:1

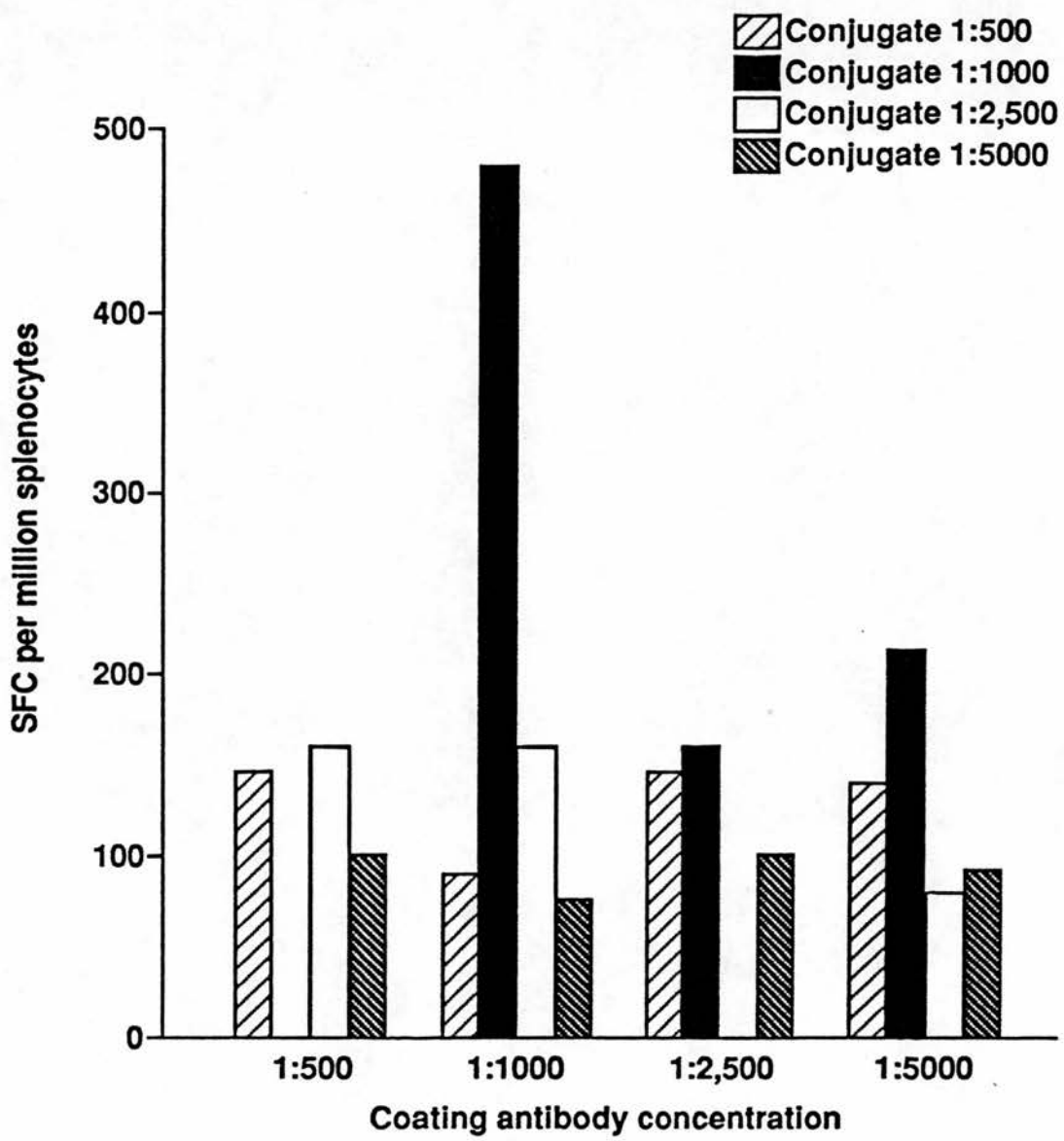
OPTIMUM COATING ANTIBODY AND CONJUGATE CONCENTRATIONS FOR THE DETECTION AND ENUMERATION OF IgA SECRETING CELLS



The best results were obtained with coating concentrations of 1:1000 and conjugate concentration 1:1000

Graph 6B:2

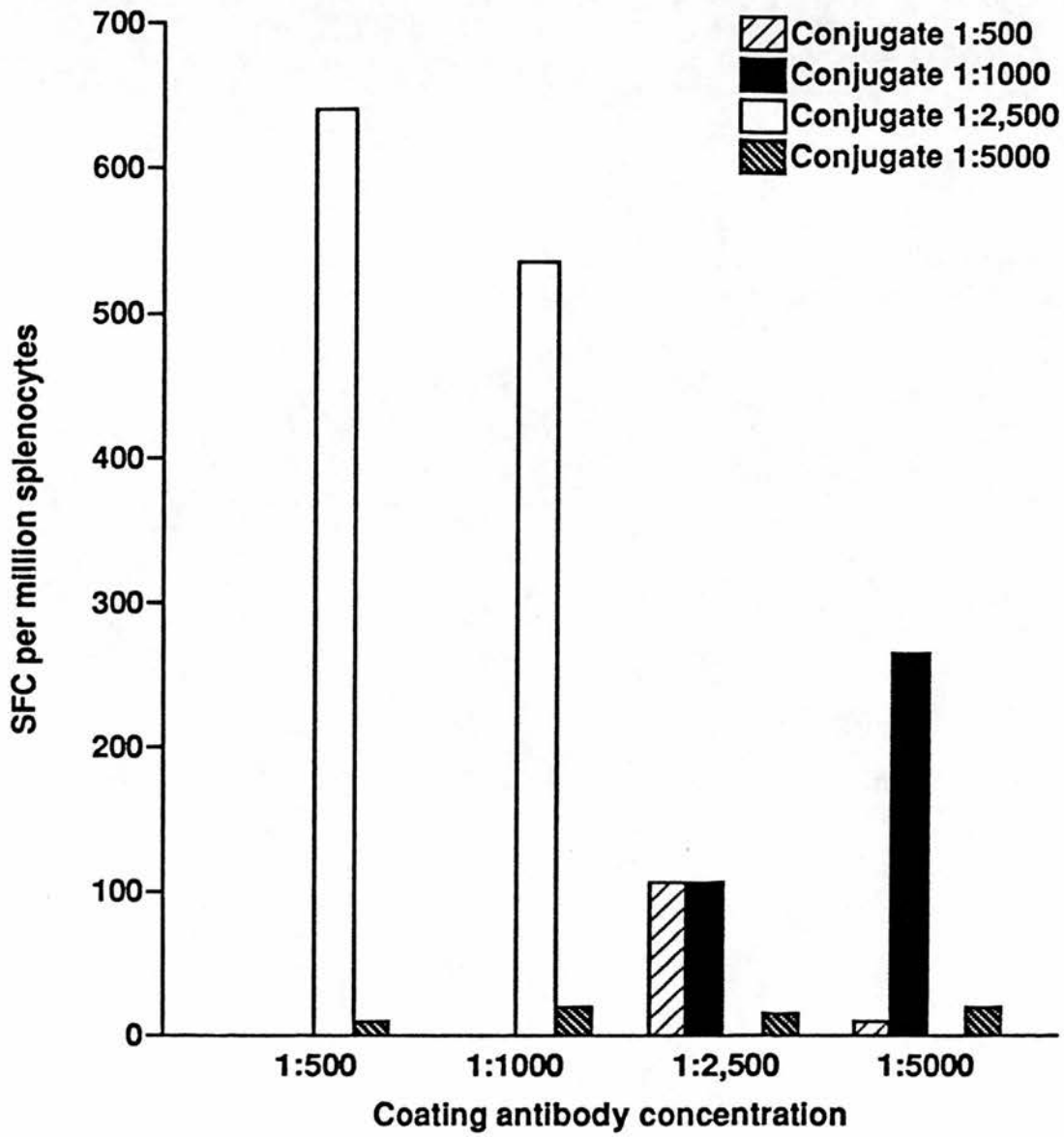
OPTIMUM COATING ANTIBODY AND CONJUGATE CONCENTRATIONS FOR THE DETECTION AND ENUMERATION OF IgM SECRETING CELLS



The best results were obtained with a coating concentration of 1:1000 and conjugate concentration 1:1000.
SFC = spot forming cells (immunoglobulin secreting cells)

Graph 6B:3

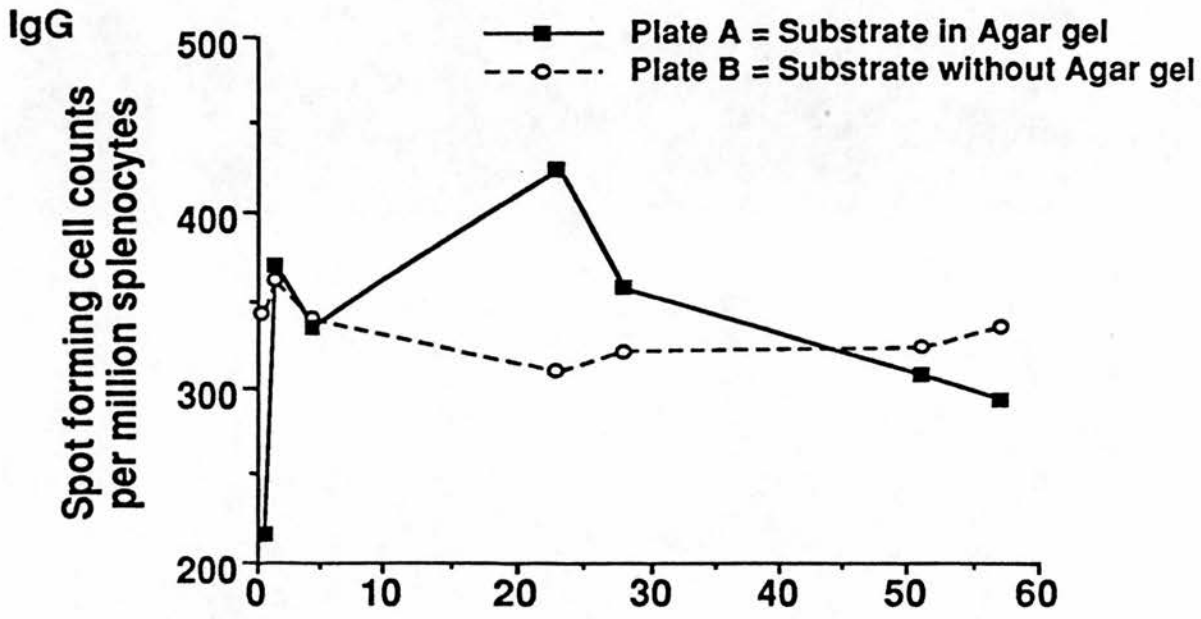
OPTIMUM COATING ANTIBODY AND CONJUGATE CONCENTRATIONS FOR THE DETECTION AND ENUMERATION OF IgG SECRETING CELLS



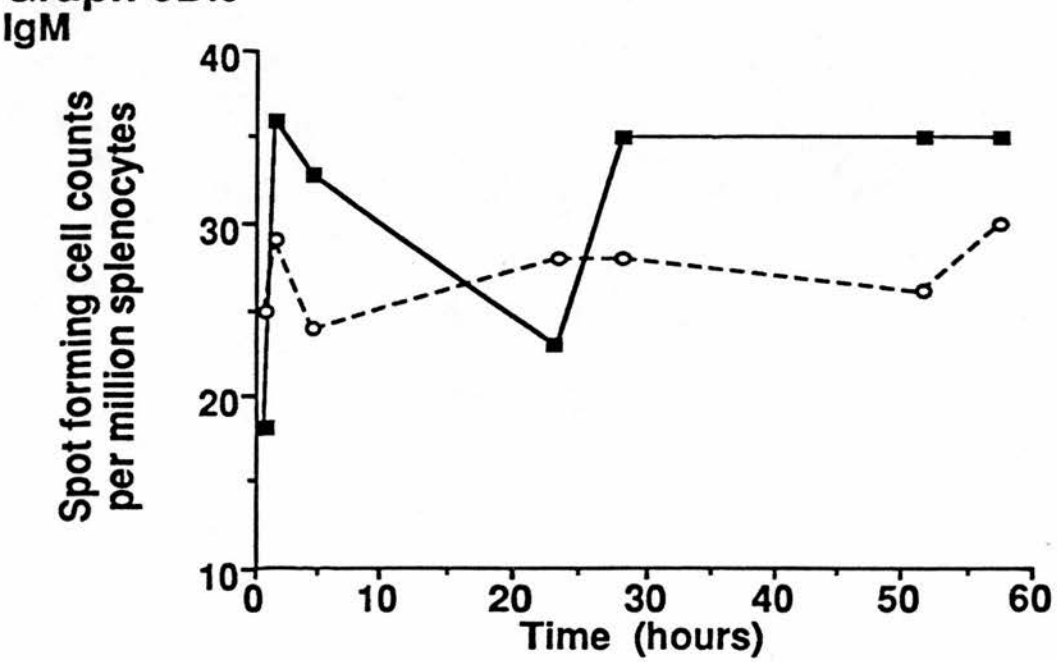
The best results were obtained with a coating concentration of 1:500 and conjugate concentration 1:2,500

Graph 6B:4

VARIATION OF THE NUMBER OF SPOTS COUNTED WITH TIME AFTER ADDITION OF SUBSTRATE (IgG and IgM anti β lactoglobulin)



Graph 6B:5



IgG - Substrate in agar gel gave higher variation in the counts (330±66) as compared to substrate without agar gel (334±17)

IgM - Substrate in agar gel gave higher variation in the counts (31±7) as compared to substrate without agar gel (27±2)

SECTION 6C:

ELISPOT AND ELISA IN THE STUDY OF MICE

REARED ON GLUTEN-FREE OR NORMAL DIET

SECTION 6C

ELISPOT AND ELISA IN THE STUDY OF MICE REARED ON GLUTEN-FREE OR NORMAL DIET

INTRODUCTION

There has been published work suggesting that mice exhibit oral tolerance to wheat gliadin, a normal dietary constituent. When compared with mice from a colony maintained on a gluten-free diet, animals taking a conventional mouse diet (CRM(X), containing 2.8% gliadin) had significantly suppressed serum IgG antibody levels and delayed type hypersensitivity responses at three weeks after parenteral immunisation with gliadin (Troncone and Ferguson, 1988).

It is not established as to whether these differences may manifest earlier depending on the sensitivity of the test and how other immunoglobulin isotypes are affected. This experiment was aimed at addressing the following questions;

1. What is the isotype distribution of the serum antibody and the changes of serum antibody levels in the early phases of the immune response?
2. How early does the difference in the serum antibody response begin to show and when does it become significant?

3. Can these differences be detected earlier by ELISPOT than by ordinary ELISA? The hypothesis was that differences would be manifest earlier b ELISPOT than ordinary ELISA as the former is not subject to dilutional effects.

EXPERIMENTAL PROTOCOL

After baseline studies in unimmunised mice age-matched female BALB/c mice from the gluten free colony (GFD) and normal diet mice of the same strain received i.p immunisation with gliadin in CFA on day 1 (as described previously). On day 7, 14, and 21 after immunisation 6 animals from each group were sacrificed (as described previously). Blood for the ELISA studies was taken from the subclavian arteries of sacrificed mice and splenocytes isolated (as described previously) for ELISPOT assay. A booster immunisation without CFA was administered on day 21 and the final 6 animals from each group sacrificed on the 28th day and studied as above.

RESULTS

Antigliadin antibodies in serum

There was no difference in the base line antibody levels. During the course of the experiment mice reared on the GFD had higher levels of antigliadin antibodies of all classes compared with the normal diet reared mice. This difference became significant by ELISA at two weeks post-immunisation for antigliadin IgA antibodies (Graph 6C:3) and by the third week for the corresponding IgM and IgG antibodies (Graphs 6C:1-2). The significant difference was maintained for IgA and IgM antigliadin antibodies even a week after the booster dose.

Antigliadin spot forming cells

There was no difference in the baseline numbers of spot forming splenocytes. During the course of the experiment mice reared on the GFD had higher numbers of antigliadin spot-forming cells than normal diet reared mice. This difference became significant within the first 7 days post-immunisation for IgM (Graph 6C:4) and 14 days post-immunisation for IgG (Graph 6C:5). IgA antigliadin antibody secreting splenocytes were not detected despite earlier successful detection in single cell suspensions

from intestinal mucosa (NOT SHOWN).

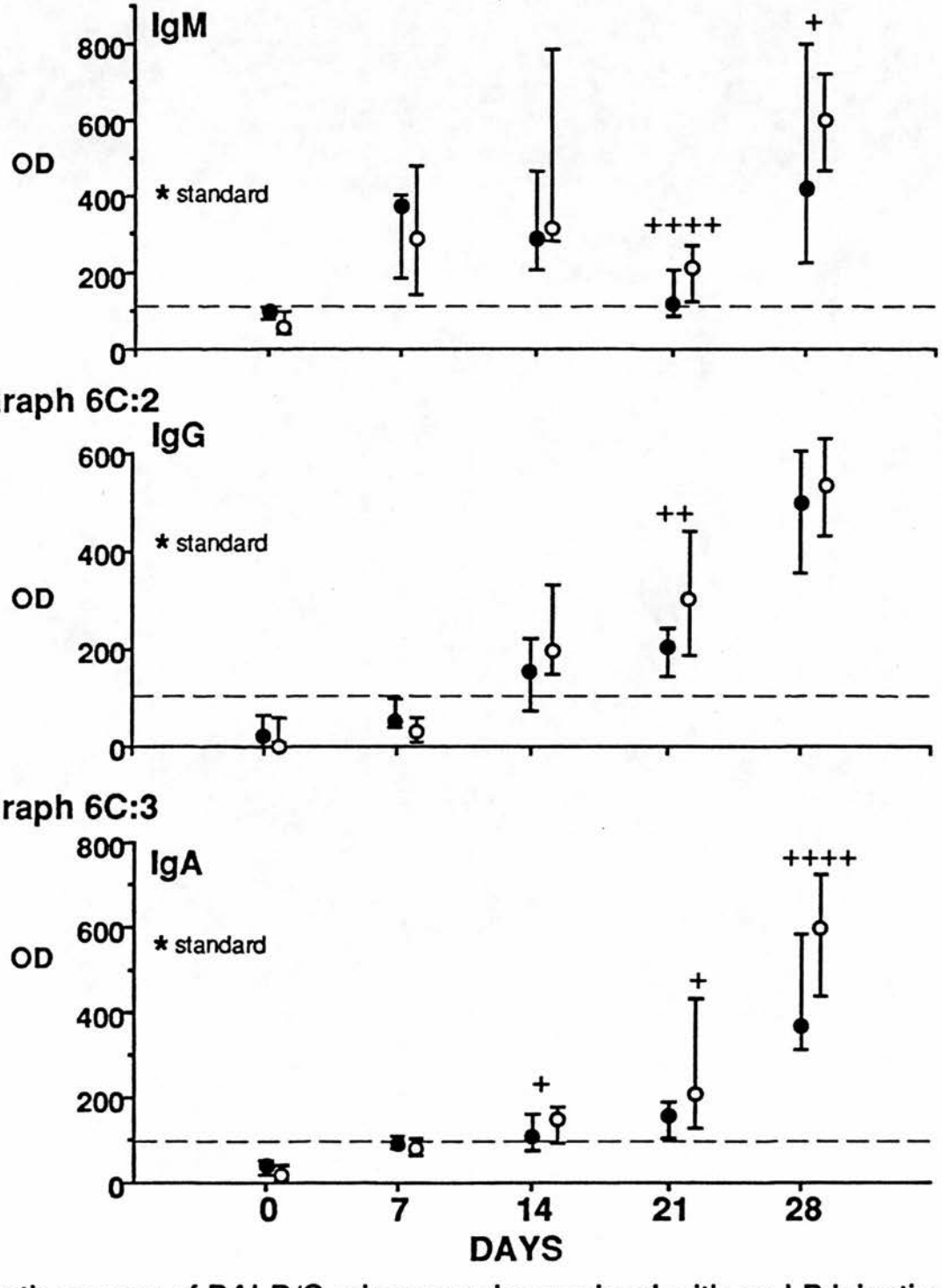
COMMENT

This work showed that mice raised on a normal, gliadin-containing, mouse diet have specific suppression of their responses to parenteral gliadin immunisation in agreement with previous work (Troncone and Ferguson, 1988).

The earliest this tolerance can be demonstrated is by the second week using ELISA (Graph 6C:3) and as early as 7 days using ELISPOT assay (Graph 6C:4). The ELISPOT assay detects antibody at the point of secretion rather than diluted in serum. This finding that the ELISPOT assay is more sensitive than ordinary ELISA is in agreement with other reports (Jannemieke et al., 1988; Lee et al., 1989). This test therefore may be more useful in detecting early changes in antibody secretory activity of B cells than ordinary ELISA. In this thesis the ELISPOT assay was used to compare food antibody activity in patients before and on elemental diet.

Graph 6C:1

THE KINETICS OF ANTIGLIADIN ANTIBODY TITRE LEVELS IN •NORMAL DIET and ○GFD REARED BALB/C MICE AFTER PARENTEREAL IMMUNISATION WITH GLIADIN IN CFA

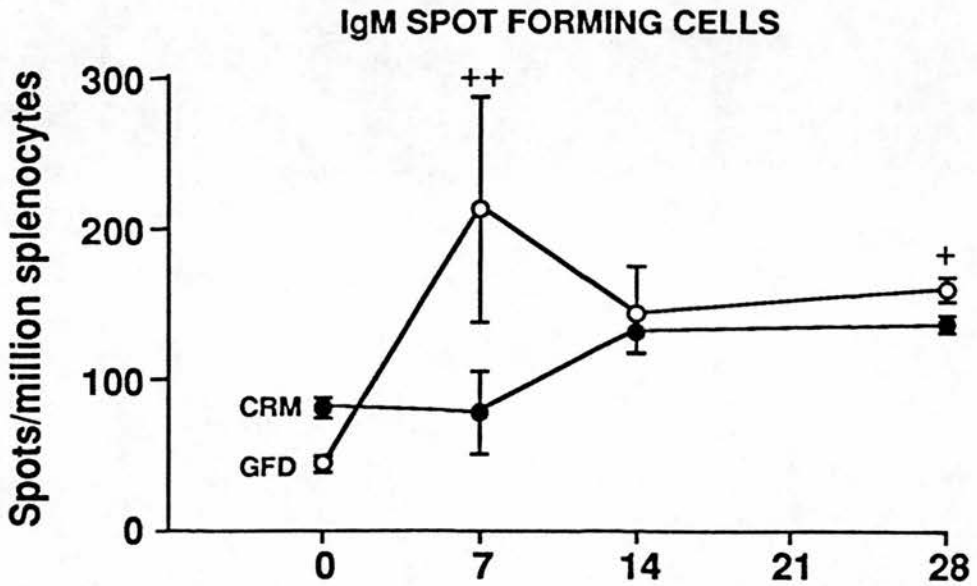


Both groups of BALB/C mice were immunised with an I.P injection of Gliadin in CFA on day 0. Serial weekly ELISA for antigliadin antibodies were performed.

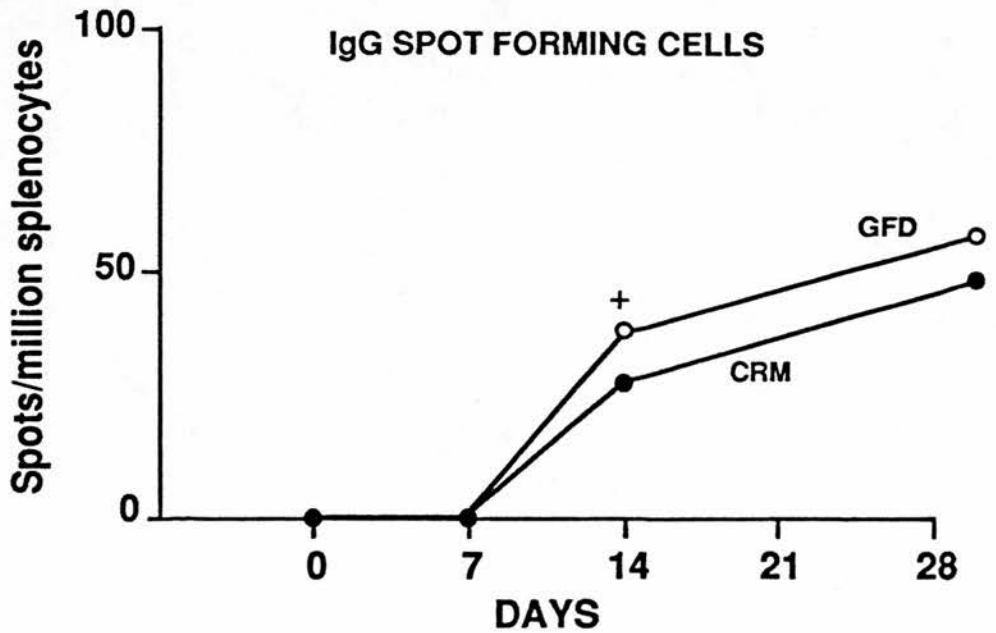
+ $p<0.05$ ++ $p<0.01$ +++ $p<0.001$ ++++ $p<0.0001$

Graph 6C:4

THE KINETICS OF ANTIGLIADIN ANTIBODY SECRETING SPLENOCYTES IN • NORMAL DIET AND ○GFD REARED BALB/C MICE AFTER PARENTEREAL IMMUNISATION WITH GLIADIN



Graph 6C:5



Both groups of BALB/C mice were immunised with an IP injection of Gliadin in CFA on day 0. Serial weekly ELISPOT assays were performed. + $p<0.05$ ++ $p<0.01$

CHAPTER SEVEN:

GUT LAVAGE FLUID PROTEINS

AND DISEASE ACTIVITY IN INFLAMMATORY BOWEL DISEASE

INTRODUCTION

The Crohn's Disease Activity Index (CDAI), developed in the 1970s (Best et al., 1976), has proved a reliable and remarkably reproducible measure of health status in patients with Crohn's disease (CD). In theory, the CDAI is vulnerable to criticism because it is heavily weighted by subjective factors of general well-being and abdominal pain, and investigators have argued that the CDAI is not so much a measure of disease activity as of general disability (Bartholomeusz and Shearman, 1989).

Many factors apart from intestinal inflammation will contribute to the index, including infection, obstruction, malnutrition, psychological dysfunction and the side effects of drugs. Nevertheless, the CDAI, and similar indices for ulcerative colitis (UC) activity such as the Powell-Tuck index (PTI) (Powell-Tuck, Bown and Lennard-Jones 1978), provide standards for the assessment of any new approach to the measurement of disease activity in IBD.

Whole gut lavage is now widely used as a bowel preparation for barium enema, endoscopy, or colorectal surgery (DiPalma et al., 1984). The clear fluid passed after initial colonic cleansing is essentially a gut perfusate.

Fluid obtained by whole gut lavage normally contains traces of IgG, albumin and alpha-1-antitrypsin; higher

concentrations are found in patients with IBD (Gaspari et al., 1988; Florent et al., 1981; Boirivant et al. 1991). In two separate studies of patients with active IBD concentrations of IgG were significantly higher than those of controls (Gaspari et al., 1988; O'Mahony et al., 1990). There was also high concentrations of WGLF albumin, with a positive correlation between lavage IgG and albumin content, suggesting that these tests are measuring plasma leakage across inflamed mucosa (O'Mahony et al., 1991b).

AIM OF THIS STUDY

The aims of this study were to establish whether assay of WGLF proteins could be used as a diagnostic test for IBD and to assess to what extent concentrations of proteins in WGLF parallel the global indices, CDAI and PTI? Do the results of this make it possible to grade severity within "active" disease, or to detect minimal abnormalities in patients with CDAI at 150 or below, or PTI at 4 or below (i.e. classified as inactive)? Does this approach offer any advantage over conventional clinical tests (ESR, C-reactive protein, platelet count), or a physician's global assessment (for the purpose of this investigation, recorded on a VAS - as described below)?

SUBJECTS AND PROTOCOL

A total of 46 patients with IBD were studied, 28 with CD (11 male, 17 female), 18 with UC (8 male, 10 female). The diagnosis and disease extent in all patients were confirmed by histology, endoscopy and radiology. Full details are given in Table 7.1 (in appendix to this chapter). The patients were classified into various sub-groups by the nature of previous resections and current macroscopic disease. Five CD patients and one UC patient were studied twice, and one man with UC was studied three times.

Patients completed a diary card of symptoms for the week preceding the lavage. Haematological and biochemical indices included haemoglobin, platelet count, ESR, C-reactive protein, total protein, albumin and serum immunoglobulins. All patients were clinically assessed by one physician, and the CDAI or PTI was calculated in the standard fashion. In addition, the physician's assessment of overall disease activity for the day of lavage was recorded on a VAS, this being based on symptoms, signs and results of the blood tests.

Whole gut lavage fluid (WGLF) was obtained from all the subjects and processed as described in chapter 6A. ELISA was performed for total IgG and total lavage albumin and lavage A1AT assayed by an immunoturbimetric method as for α-1AP (see Methods Chapter 6A).

Correlation coefficients were calculated by using the Spearman Rank Correlation test.

RESULTS

The patients studied were heterogeneous in disease distribution, and in their previous and current treatment. In CD, the CDAI range was from zero to 321; 24 lavages were in patients with CDAI of >150 (described below as having active disease) and 9 in patients with a CDAI ≤ 150 (in remission). In patients with UC, the PTI ranged from zero to 13; 13 lavages were in patients with active disease (PTI of >4) and 8 were in patients in remission with a PTI of ≤ 4.

WHOLE GUT LAVAGE FLUID PROTEIN CONCENTRATIONS

Reference ranges for WGLF IgG, albumin and A1AT have previously been established by studies of 63 immunologically normal subjects: 20 men, age range 15-80 and 43 women, age range 24-88, all taking a normal diet, none receiving immunosuppressive or anti-inflammatory drugs (Brydon, Choudari and Ferguson submitted to gut). Normal values are for IgG ≤ 10 ug/ml, for albumin ≤ 26 ug/ml, and for A1AT ≤ 19 ug/ml.

Values for WGLF protein concentrations in CD and UC patients are shown in Figure 7.1, plotted against CDAI or

PTI as appropriate.

DIAGNOSTIC POTENTIAL OF WHOLE GUT LAVAGE FLUID TESTS

If WGLF protein concentrations are always high when there is overt intestinal mucosal inflammation (on radiological or endoscopic visualisation), this would provide a means of screening patients for the presence or absence of IBD. This issue has been considered by analysis of the results for WGLF IgG. High values for IgG concentration were present in 24 of the 27 lavages performed in patients who had unequivocal macroscopic small bowel or colonic CD, and in 13 of the 15 lavages in patients with UC who had positive X-ray or endoscopic findings. There was also one patient with microscopic evidence of colonic CD, no macroscopic disease but high WGLF IgG concentration.

WHOLE GUT LAVAGE FLUID PROTEINS AND DISEASE ACTIVITY

IgG, CROHN'S DISEASE

In 33 lavages from patients with CD, values for WGLF IgG concentration were normal in 9. Seven of these were in patients in remission and one in an 18-year old man with a borderline CDAI of 168. He had ankylosing spondylitis, and his anorexia and high ESR shortly after right hemicolectomy for CD were thought to reflect activity of

arthritis rather than of CD. There was one patient with WGLF IgG concentration of 10 ug/ml and CDAI of 185. Abnormally high concentrations of WGLF IgG were present in 24 cases. Twenty-two had active disease; one was an obese woman with oro-facial CD and a CDAI of zero, mainly due to a high weight for height value; her WGLF IgG was 12ug/ml. A 13 year-old boy with perianal CD and unexplained abdominal pain had a CDAI of 119 and WGLF IgG was 20ug/ml. During the next six months his growth stopped and pain became worse; both problems have resolved with systemic steroid therapy.

IgG, ULCERATIVE COLITIS

In 21 lavages from patients with UC, values for WGLF IgG concentration were normal in 8 cases (of whom 7 were in remission) and high in 13 cases (12 with active disease). Both discrepant cases were patients who had had unequivocally active disease two weeks before and were in the early stages of sulphasalazine or oral corticosteroid treatment respectively.

ALBUMIN AND IBD

In 33 lavages from patients with CD, values for WGLF albumin concentration were normal in 16 cases (of whom 9 were in remission, CDAI in the others ranging from

168-283). Albumin concentrations were high in 17 cases, all with active disease.

In 21 lavages from patients with UC, values for WGLF albumin concentration were normal in 12 cases, (of whom 8 were in remission); and were abnormally high in 9 cases, all with active disease.

ALPHA-1-ANTITRYPSIN AND IBD

In 33 lavages from patients with CD, values for WGLF A1AT concentration were normal in 18 cases (of whom 8 were in remission), and were high in 15 cases (14 with active disease).
In 21 lavages from patients with UC, values for WGLF A1AT concentration were normal in 14 cases (of whom 7 were in remission), and high in 7 cases, (6 with active disease).

COMMENT

These results show that these indices do not discriminate between the presence and the absence of disease. Normal values were found in patients with clinically inactive disease but with definite radiological and macroscopic evidence of IBD.

CORRELATION OF LAVAGE PROTEINS CONCENTRATIONS WITH ACTIVITY INDICES IN ACTIVE AND INACTIVE DISEASE

Correlation co-efficients for WGLF protein concentrations against activity indices are detailed in Table 7.2 (in appendix). For both CD and UC, and for all three proteins studied, there was a highly significant positive correlation between activity index and WGLF protein concentration, with r values ranging from 0.439 to 0.714. Since, as shown clearly in Figure 7.1, the relationship between activity index and lavage protein concentration is not linear but biphasic, correlations were separately calculated for cases with active and inactive disease (as defined by CDAI and PTI). Calculations were also performed for the subsets of CD patients with active colonic and active small bowel disease.

The results (Table 7.2 in appendix) show that only in active disease are there significant correlations; that the highest r values were found for IgG versus CDAI in active CD (r=0.821), and particularly in the subset with active small bowel CD (r=0.970); and that in active UC, although there was good correlation between WGLF IgG concentration and the PTI (r=0.740), the other two proteins did not show significant correlation with the PTI. In inactive CD and UC, WGLF protein concentrations were independent of activity indices.

CONVENTIONAL LABORATORY INDICES OF DISEASE ACTIVITY

For the purposes of this analysis, patients with CD who had a CDAI of >150, and those with UC who had a PTI of >4, have been designated as having active disease. The numbers of cases with abnormal and normal values for ESR, C-reactive protein and platelet count in the active and remission groups are given in Table 7.3 (in appendix). This table also includes, for comparison, numbers with normal and abnormal WGLF IgG, albumin and A1AT values, and the physician's overall assessment based on a VAS.
The three blood indices were normal in most patients with inactive disease. However they were also normal in many patients with unequivocally active disease, and this was particularly so in the group of 13 assessments in active UC - ESR was normal in 8 cases, C-reactive protein was normal in 10 and platelet count was normal in 8.

GENERAL DISCUSSION

There have been many attempts to develop a single index of illness in IBD (Best et al., 1976; Powell-Tuck et al., 1978; Truelove and Witts, 1955; Harvey and Bradshaw, 1980; Van Hees et al., 1980; De Dombal et al., 1974; Myren et al., 1984; Sandler et al., 1988) by combining a few clinical and/or laboratory criteria, and claims have been made for the significance of single laboratory

determinations in some situations (Sachar et al., 1986; Fegan et al., 1982; Jansen et al., 1976; Meyers et al., 1985 and Harries et al., 1983). This was an alternative approach, attempting to separate and independently measure the various components of disability in IBD so that those which are potentially treatable by medical, anti-inflammatory regimes can be considered separately from those which require other approaches.

Investigation of gut immunity requires direct study of tissues and secretions, and thus presents major logistic and ethical problems if serial in vivo studies are to be undertaken. Exploitation of the relatively new method of bowel cleansing by whole gut lavage has considerable potential as a source of material for clinical evaluation of intestinal immunity and inflammation (Gaspari et al., 1988).

There have been reports of high concentrations of IgA but only trace amounts of IgG (<5ug/ml) in fluid from control subjects, whereas there were significantly higher IgG concentrations in WGLF from patients with active Crohn's disease (Gaspari et al., 1988). In another study this was accompanied substantial amounts of IgG in WGLF indicating that these tests may be detecting plasma leakage into the gut (O'Mahony et al., 1990). The assay of A1AT, was included in the protocol in view of its widespread acceptance as an index of GI protein loss (Meyers et al., 1985).

The aims of this study were to determine whether assay of WGLF proteins can be used as a diagnostic test for IBD, and whether this completely objective procedure can substitute for, or complement, currently used clinical tests and indices of disease activity. The best currently available index, the CDAI, was used as the reference measure of disease activity in CD, and a similar index for UC, the PTI.

This study shows that WGLF protein concentrations, particularly IgG, discriminate well between active and inactive IBD; and also, within the subsets of patients with active disease, closely parallel disease activity as defined by CDAI and PTI. Furthermore, these sensitive tests were completely normal in most patients with CDAI <150 and PTI <5, strongly supporting the present clinical trials practice whereby such values are taken as successful end-points, indicating that remission has been achieved.

However WGLF protein assays cannot be used as screening or diagnostic tests for IBD. Although high values are found in many patients, the data in this paper clearly show that values are normal in clinically inactive disease, even when there are unequivocal radiological or endoscopic features of IBD such as cobblestone ulcers and long, string-like strictures. Nevertheless WGLF IgG is a valuable aid to diagnosis in some clinical situations such as in patients with microscopic CD, when IBD

co-exists with severe diverticular disease, and in separating the effects of intestinal and joint disease in IBD patients with ankylosing spondylitis.

As regards the source of WGLF total IgG a few factors have to be considered. There is abundant evidence to implicate IgG in the pathological lesions of IBD, particularly CD - immunohistochemistry reveals an excess of IgG-containing cells, deep in the mucosa (Baklien and Brandtzaeg, 1976; see chapter 1); isolated intestinal mononuclear cells from IBD patients spontaneously secrete high amounts of IgG (MacDermott et al., 1981; Verspaget et al., 1988); and there are differences in intestinal plasma cell IgG subclass distribution between controls, UC and CD patients (Kett, Rognum and Brandtzaeg, 1987). Thus, the possibility arose that IgG was probably largely from the abundant IgG cells in diseased mucosa. However the findings of high concentrations of two plasma proteins, albumin and A1AT, in lavage fluid from patients with active IBD suggests that at least some of the IgG is plasma-derived. Furthermore, all three protein concentrations were low in WGLF from some inactive CD patients who have extensive chronic ulceration of the intestine (diseased tissues known to be replete in IgG cells).

It could be argued that these sensitive tests are merely measuring plasma proteins entering the gut lumen as a result of bleeding. However, in active IBD, when abundant

amounts of the three proteins are present in WGLF, their relative concentrations differ substantially from those in plasma. The three proteins differ in molecular weight, IgG 150,000 daltons, albumin 69,000 daltons, A1AT 45,000 daltons, and this suggests that there is a selective increase in mucosa-to-lumen permeability in active IBD, operating either at the level of the capillary or the epithelial basal lamina.

Although fluids are processed within minutes of collection, proteins leaking into the gut lumen proximally will have been exposed to pancreatic and bacterial proteases during the transit of fluid along the gut (1-2 hours). Ex vivo experiments show that at 37°C, degradation of albumin by unprocessed WGLF is significant (loss of 10-40% of measured albumin in one hour), whereas IgG and A1AT are relatively resistant to such proteolysis. This could explain the differences in results for IgG and albumin in active disease, but not the relative insensitivity of WGLF A1AT concentrations.

Various scientific approaches to the measurement of disease activity have recently been reviewed (Singleton, 1987; Camilleri and Proano, 1989). Laboratory tests such as ESR, platelet count, acute phase proteins, are useful. However they may be normal in active IBD, particularly in UC and in small bowel Crohn's disease (see Table 7.3) and will be positive when there is active inflammation or infection outwith the gut.

Other techniques such as labelled leucocyte studies (Saverymuttu et al., 1986; Crama-Bohbouth et al., 1988) and measures of GI protein loss by A1AT clearance (Florent et al., 1981; Boirivant et al. 1991; Strygler et al., 1990), have advantages and disadvantages which have been fully discussed elsewhere (O'Mahony et al.,1990; Singleton, 1987; Camilleri and Proano, 1989). In general, they have been developed and applied without being subjected to prospective evaluation against the carefully developed standard activity indices such as CDAI. Furthermore, labelled leucocyte studies are expensive, involve exposure to radio-activity, depend on complete faecal collection and usually require hospital admission. The findings with these tests relate to events in macroscopically affected gut and thus differ with those obtained with the gut lavage technique which appears to measure a more diffuse phenomenon.

There may be problems in interpretation of isotope-based or A1AT clearance studies if the treatment used, such as elemental diet therapy, changes intestinal transit rate or otherwise influences defaecatory function; this could either mask or mimic changes due to specific anti-inflammatory effects of the test treatment. Since the standard protocol for collection of whole gut lavage fluid is not influenced by concurrent dietary or pharmacological treatment, this method should lend itself to clinical trials.

CONCLUSION

Gut lavage is now widely used for bowel preparation prior to endoscopy, radiology and surgery. The procedure is well tolerated by patients and volunteers. Lavage fluid, collected as described above, is aesthetically acceptable to laboratory staff, resembling urine rather than faeces, and specimens can be stored at -70^{o}C for several months without deterioration. Concentration of IgG in WGLF, assayed by ELISA, discriminates very well between active and inactive IBD, and accurately grades the degree of activity. This new and direct approach to clinical investigation of gut immunity was used in the analysis of illness and of response to treatment in this thesis and has considerable potential in clinical trials of anti-inflammatory and immunomodulatory regimens.

APPENDIX FOR CHAPTER 7

TABLE 7.1 CLINICAL DETAILS OF 46 PATIENTS WITH IBD, UNDERGOING WHOLE GUT LAVAGE ON 54 OCCASSIONS

CROHN'S DISEASE

Parts of GI tract Previously resected	Number of Patients	Macroscopic disease at time of lavage	Number of lavages
None	16	oro-facial	1
		small bowel	6
		ileocolonic	2
		colonic	4
		rectosigmoid	3
		peri-anal	2
		*microscopic	1
Small bowel	2	small bowel	2
Ileo-caecal ±terminal ileum	8	no macroscopic disease	5
		small bowel	3
		ileocolonic	1
Segmental colonic resection	2	ileocolonic	1
		colonic	2
Total, Crohn's disease	**28**		**33**

ULCERATIVE COLITIS

Previously documented macroscopic disease			
Recto sigmoid	11	no macroscopic disease	3
		recto-sigmoid	9
Left sided	3	no macroscopic disease	1
		left sided	2
pancolitis	4	no macroscopic disease	1
		pancolitis	5
Total, ulcerative colitis	**18**		**21**

*Microscopy - no macroscopic disease detected

TABLE 7.2 Concentrations of proteins in WGLF from patients with active and inactive IBD; correlations with CDAI and PTI

	Number of lavages	IgG concentration vs activity index		Albumin concentration vs activity index		A1AT concentration vs activity index	
		r	p	r	p	r	p
Crohn's disease							
All	33	0.697	<0.0001	0.590	<0.0001	0.439	<0.01
Active	24	0.821	<0.0001	0.547	<0.01	0.293	NS
Inactive	9	-0.022	NS	-0.180	NS	0.105	NS
Active colonic	8	0.778	<0.05	0.507	NS	0.468	NS
Active small bowel	10	0.970	<0.0001	0.547	NS	0.359	NS
Ulcerative colitis							
All	21	0.714	<0.0001	0.701	<0.0001	0.527	<0.02
Active	13	0.740	<0.01	0.610	<0.05	0.600	<0.05
Inactive	8	-0.318	NS	-0.378	NS	-0.041	NS

NS - not significant, P>0.05

FIGURE 7.1 Correlation of Lavage Proteins Concentrations with Activity Indices in Active and Inactive Disease.

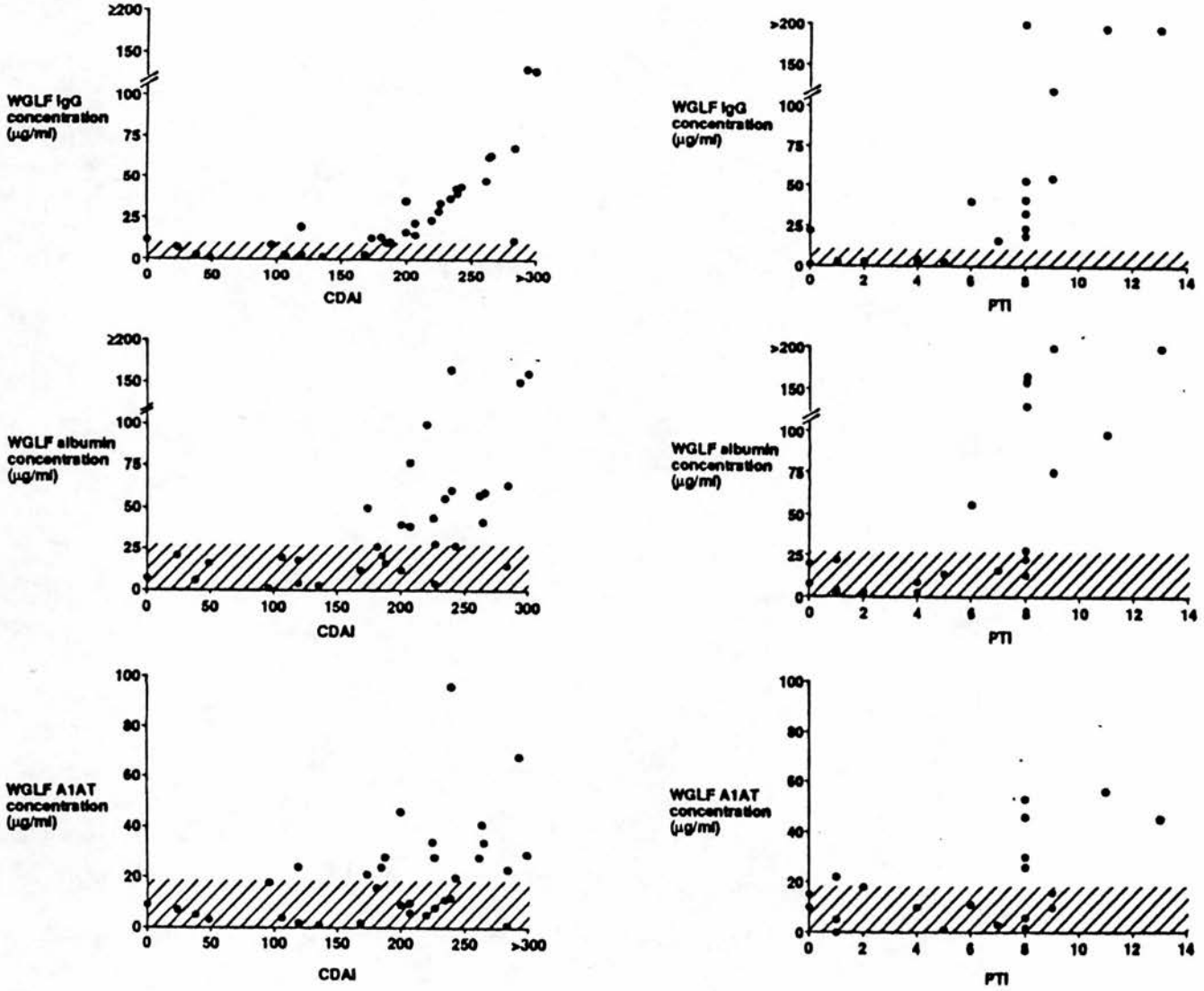


TABLE 7.3 Presence of normal or abnormal values for conventional laboratory indices of disease activity, global assessment, and WGLF IgG, albumin and A1AT concentrations in patients with Crohn's disease and ulcerative colitis, arbitrarily subdivided into active or inactive IBD on the basis of CDAI or PTI respectively

	Number of lavages	ESR		C reactive protein		Platelet count		Visual analogue scale	
		normal <20	high >20	normal <1.5	high >1.5	normal	high	normal 0-60	high 61-120
Crohn's disease									
Active (CDAI >150)	24	5	19	8	16	6	18	1	23
Inactive (CDAI <150)	9	6	3	9	0	8	1	8	1
Ulcerative colitis									
Active (PTI>4)	13	8	5	10	3	8	5	2	11
Inactive (PTI<4)	8	8	0	8	0	7	1	6	2

TABLE 7.3 Continued

WGLF IgG concentration		WGLF albumin concentration		WGLF A1AT concentration	
normal ≤10	high >10	normal ≤26	high >26	normal ≤19	high >19
2	22	7	17	10	14
7	2	9	0	8	1
1	12	4	9	7	6
7	1	8	0	7	1

CHAPTER EIGHT:

SECRETORY IMMUNOGLOBULIN A LEVELS IN IBD AND CONTROLS

INTRODUCTION

There are reports that IBD patients have higher levels of IgA in intestinal secretions than normal controls (see chapter 2A). The importance of IgA to mucosal surfaces both in terms of immune protection and down-regulating inflammatory responses is well established (Tomasi et al., 1965; Eddie et al., 1971), and was reviewed in chapter 2A.

AIM OF THIS STUDY

The aims of the experiments described in this chapter were as follows;

1. To establish the pattern of total IgA, non-secretory IgA and secretory IgA immunoglobulins in WGLF of patients with IBD and controls and to define similarities or differences if any.
2. To find out whether there are any changes in the levels of total IgA, non-secretory IgA and secretory IgA corresponding with changes in disease activity.

SUBJECTS AND PROTOCOL

Details of the study population are listed in (Table 8:1 in the appendix to this chapter). Whole gut lavage fluid was collected from 60 patients (35 Crohn's disease and

25 ulcerative colitis) and also from 16 controls. The diagnosis and disease extent in all patients were confirmed by histology, endoscopy and radiological criteria. The median age of the patients with Crohn's disease was 46 with a range of 14-83. The median age for the ulcerative colitis patients was 47 with a range of 24-82.

Disease activity was based on clinical assessment and WGLF total lavage IgG concentration (see chapter 7).

At the time of lavage 20 of the Crohn's disease patients had active disease, and 15 had inactive disease. The macroscopic manifestations of the disease were as follows: orofacial (1 patient), jejunal (5), terminal ileum (3), ileocaecal (12), colonic (4), rectal (5), perianal (4). One patient had microscopic colitis.

Drug treatment in this group was as follows: twelve were on oral steroid with a daily dose ranging between 10mg and 40mg, 7 were on salazopyrine, 2 on 5 amino salicylates and 14 on no drug treatment at all for their Crohn's disease.

At time of lavage, 16 of the ulcerative colitis patients had active disease and 9 had inactive disease. The macroscopic involvement in patients with ulcerative colitis was as follows: pancolitis (8), left-sided colitis (5), proctitis (8) and pouchitis. One patient had microscopic colitis. Eleven were on no drug treatment, 3 were on oral prednisolone and salazopyrine, 5 on

salazopyrine only, 3 on an amino-salicylate type drug plus rectal steroids, and one on a combination of cyclosporin, oral steroids and colifoam enema.

The controls were healthy volunteers and subjects who after investigation were found to have irritable bowel syndrome or other non-inflammatory disease conditions. The final diagnoses in the controls were as follows: non-inflammatory polyps in the colon (2),irritable bowel syndrome (7), and one each with colonic angiodysplasia, duodenal ulcer, delusions, colonic cancer and abdominal pain. There were two normal volunteers. The median age was 47 years with a range of 21-79. All the subjects were on a normal diet.

Whole gut lavage fluid (WGLF) was obtained from all the subjects and processed as described in chapter 6. ELISA for total IgA and secretory IgA (sIgA) as described in chapter 6 was performed simultaneously on the same plates for each patient. Data were analysed to compare active and inactive disease groups and controls in order to ascertain firstly if there was any difference between disease groups and controls and secondly whether the differences were increased with higher disease activity.

RESULTS

LEVELS OF SECRETORY IgA AND THE PROPORTION OF SECRETORY IgA TO THE TOTAL IgA IN WGLF

There was no significant difference in the levels secretory IgA between the groups (Table 8:2 in appendix). Patients with active IBD had a higher median value than controls or patients with inactive IBD, however this was not statistically significant.

THE PROPORTION OF SECRETORY IGA

This was the expression of sIgA as a percentage of the total IgA in WGLF and was derived for each specimen as follows;

$$\frac{\text{WGLF sIgA concentration}}{\text{WGLF IgA concentration}} \times 100$$

Differences in the proportions of sIgA between controls and disease groups would indicate alteration in the assembly of sIgA which involves 'J' chains and the secretory component in the disease groups or increased leakage of serum derived IgA into intestinal secretions in the disease groups. Distinction between these two mechanisms would not be possible with this experiment but

if the differences are well demarcated they could be used as a marker of disease activity.

Control WGLF proportion of sIgA gave a median of 100% with a range of 7-117 (Table 8:2). All IBD patients as a group gave a median of 69, range 4-107 significantly lower than control values (p=0.003). When the IBD patients were further divided into active and inactive disease groups, the active IBD group gave a range of 4-97 with a median of 67, while the inactive IBD group had a median of 72 with a range of 40-107. Both these results were significantly lower than for the controls at p=0.0028 for the active group and p=0.0244 for the inactive group.

THE PROPORTION OF sIgA IN CROHN'S DISEASE and ULCERATIVE COLITIS SUBGROUPS

The proportions of sIgA were significantly lower for both Crohn's disease (p=0.0088) and ulcerative colitis (p=0.0059) compared to controls (Table 8:3).

Among the Crohn's disease patients, the subgroup with active disease had significantly (p=0.0130) lower proportions of sIgA than controls (Table 8:4). The significance of the difference between controls and inactive Crohn's disease (p=0.0459) was borderline.

The group of patients with active ulcerative colitis had a significantly lower percentage of sIgA compared to

controls (p=0.0067); the proportions of sIgA in the patients with inactive ulcerative colitis did not differ significantly from the control group (p=0.0842).

LEVELS OF NON-SECRETORY IgA IN WGLF

The concentration of non-secretory IgA was derived by subtracting secretory IgA concentration from total IgA. Controls had very low levels of non-secretory IgA in WGLF, giving a median of 0 with a range of -35 to 860 (The minus value comes from a few results where secretory IgA assay figures were higher than total IgA). The difference in the levels of non-secretory IgA between the controls and active IBD was significant (p= 0.0008) and also inactive IBD had significantly higher (p=0.0169) levels than controls (Table 8:5).

When Crohn's disease and ulcerative colitis groups were compared to normal controls the differences were significant for both Crohn's disease group (p=0.0041) and ulcerative colitis group (p=0.0021). This difference held for the active Crohn's disease group (p=0.0040) and active ulcerative colitis group (p=0.0029) whereas for both inactive Crohn's disease (p=0.0459) and inactive ulcerative colitis (p=0.0445) the significance was borderline (Table 8:6).

THE PROPORTION OF NON-SECRETORY IgA IN WGLF OF IBD PATIENTS

This was the expression of non-secretory IgA as a percentage of the total IgA in WGLF and was derived for each specimen as follows;

$$\frac{\text{WGLF nsIgA Concentration}}{\text{WGLF IgA concentration}} \times 100$$

The proportion of secretory IgA was negligible in the controls; a median of 0 with a range of -17 to 93. Both active IBD ($p=0.0031$) and inactive IBD ($p=0.0272$) had significantly higher proportions than controls (Table 8:7A). Further subdivisions into Crohn's disease and ulcerative colitis similarly gave significantly higher proportions than controls at $p=0.0105$ and $p=0.0.0059$ respectively. With respect to disease activity the proportions of nsIgA were significantly higher for active Crohn's ($p=0.0149$) and active ulcerative colitis ($p=0.0067$). Both inactive ulcerative colitis ($p=0.0842$) and inactive Crohn's disease ($p=0.0528$) gave proportions of nsIgA not significantly higher than controls (Table 8:7B).

COMPARISONS OF SECRETORY IgA LEVELS (sIgA), THE PROPORTION OF SECRETORY IgA (%sIgA), NON-SECRETORY IgA (nsIgA) AND THE PROPORTION OF NON-SECRETORY IgA (%nsIgA) BETWEEN DISEASE GROUPS

Comparisons were made between disease groups for levels of sIgA, nsIgA and the proportions of sIgA (%sIgA) and nsIgA (%nsIgA). There was no difference between active IBD and inactive IBD disease groups nor was there any significant difference between Crohn's disease patients and patients with ulcerative colitis. Even when the disease groups were divided further into active and inactive groups, there was no significant difference between corresponding active CD and active UC or inactive CD and inactive UC. Neither did all the parameters differ significantly between active CD and inactive CD patients or between active UC and inactive UC patients (Tables 8:8A and 8:8B).

RELATIONSHIP BETWEEN TOTAL IgA AND SECRETORY IgA

In IBD patients as a whole, total IgA correlated strongly ($r=0.806$, $p < 0.0001$) with secretory IgA (Table 8:9A). This was also the case among the various disease subgroups, the correlation coefficient ranging from 0.690 for the active ulcerative colitis group to 0.945 for the inactive Crohn's disease group (Tables 8:9B).

The higher the total IgA the lower the proportion of secretory IgA compared to the total (%sIgA); the correlations between total IgA and proportion of secretory IgA were all negative (r=-0.231 to -0.646), except for the inactive UC group (r=0.226). These correlations were significant for all IBD patients (r=-0.388, p=0.01); and all UC patients (r=-0.231, p=0.039) [Table 8:9A]. The negative correlation between total IgA and the proportion of secretory IgA was maintained even when the IBD patients were further subdivided by diagnosis and disease activity. This correlation was significant for active UC (r=-0.478, p= 0.028) and inactive Crohn's (r=-0.646, p=0.006) [Table 8:9B].

THE RELATIONSHIP BETWEEN DISEASE ACTIVITY AND SECRETORY IgA

Disease activity was assessed by the total amount of IgG in WGLF (chapter 6). Total IgG significantly correlated with secretory IgA for all groups except the following; controls, inactive IBD, inactive Crohn's disease, all patients with ulcerative colitis (Table 8:10).
There was no significant correlation between the level of IgG in WGLF and the percentage of secretory IgA except in the controls (r=-0.225,p=0.001) where the higher the IgG the lower the proportion of secretory IgA (Table 8:11).

THE RELATIONSHIP BETWEEN NON-SECRETORY IgA AND DISEASE ACTIVITY

The level of non-secretory IgA in lavage correlated with lavage IgG ($r=0.241$, $p < 0.0001$) for the IBD patients taken as one group. Active IBD patients, Crohn's disease, active Crohn's disease showed significant positive correlations (Table 8:11). Taken separately all ulcerative colitis groups, active or inactive did not show any significant correlation between non-secretory IgA and lavage IgG in WGLF.

The proportion of non secretory IgA in WGLF fluid was correlated with disease activity as measured by total lavage IgG for all the subject groups. There was no significant correlation for all subject groups regardless of disease activity. Potential sources of this non-secretory IgA are discussed below.

COMMENT

The aims of these experiments were to establish the pattern of IgA, sIgA and non-secretory IgA in patients with IBD compared to controls, and whether these changes corresponded with disease activity.

My results show that IBD patients have higher levels of total IgA in WGLF than controls, and that the levels of secretory IgA were directly proportional to the levels

of lavage IgA in WGLF for all the groups. Though the **absolute amounts** of secretory IgA were higher in disease groups than controls, the differences were not significant. However secretory IgA as a **proportion** of total IgA was much lower for the disease groups compared to controls. The significant difference was confined to both active Crohn's disease and ulcerative colitis groups and not the inactive disease groups.

The finding of decreased proportion of secretory IgA as part of the total IgA also agrees with previous reports. Brandtzaeg and Korsud (1984) reported that there was a decreased production of the 'J' chain by mucosal lymphocytes as well as a reduced binding capacity for secretory component by mucosal IgA in patients with IBD. The decreased secretory IgA as a proportion of total IgA could also be due to leakage of monomeric IgA from the plasma into the intestinal secretions causing dilution of the secretory IgA. The actual break in the mucosal barrier in IBD may lead to increased plasma leakage into the intestinal lumen (Saverymuttu et al., 1985a and 1985b). Serum derived IgA would be mainly monomeric IgA with a higher proportion of IgA1 which has a low affinity for the secretory component (Delacroix et al., 1982; Kett, Brandtzaeg and Fousa, 1988). These factors therefore would lead to a decrease in the proportion of secretory IgA to total IgA in IBD patients.

Both the absolute amount and the proportion of

non-secretory IgA are significantly higher in active disease than in controls. This is also the case with WGLF IgG, where the increased leakage of WGLF IgG corresponds with disease activity (see chapter 7). It is therefore probable that the source of this increased non-secretory IgA is the same as that of WGLF IgG, namely serum. However non-secretory IgA was not found to correlate consistently with disease activity. The lesser discriminating ability of non-secretory IgA compared to WGLF IgG could be because the concentration of non-secretory IgA in intestinal secretions is subject to the variable factors mentioned above (Brandtzaeg and Korsud, 1984) apart from leakage from serum whereas WGLF total IgG is mainly a leak from serum and not subject to binding by secretory piece or 'J' chain. Therefore as a marker of disease activity IgG would be a better index. This work did not look at IgA subclasses even though increased local production of IgA1 has been reported in IBD (Macdermott et al., 1986; see chapter 2B).

These factors combined most probably account for the significantly increased proportions of non-secretory IgA in active IBD.

The strong correlation between secretory IgA and IgG in WGLF, a measure of disease activity, suggests that increased secretory IgA production and secretion is a feature of disease activity in IBD.

There was no difference in the levels or the proportion

of sIgA or non-secretory IgA between disease groups (Tables 8:8A and 8B), indicating that even in quiescent disease IBD patients still have some immune dysregulation which becomes prominent with increased disease activity.

SUMMARY

These results show increased levels of total IgA in intestinal secretions of patients with IBD. Since IgA in the intestinal lumen offers mucosal protection, high levels could indicate an aberrant reaction to a normal antigenic load, or persistence of ubiquitous antigen, or an upregulation of the immune system intrinsic to IBD patients.

The proportion of the protective secretory IgA is significantly lower in patients with IBD as compared to controls. However within the IBD group the levels are not significantly different between disease groups regardless of activity and therefore it is not a useful index of disease activity.

APPENDIX FOR CHAPTER 8

TABLE 8:1 STUDY POPULATION

	CROHN'S DISEASE	ULCERATIVE COLITIS	CONTROLS
NUMBERS	35	25	16
SEX M:F	14:21	11:14	5:11
AGE			
MEDIAN	46	47	47
RANGE	14-83	24-82	21-79

DISEASE DISTRIBUTION

CROHN'S DISEASE		ULCERATIVE COLITIS	
Orofacial	1	Rectum only	8
Jejunal	5	Left sided	5
Terminal ileum	3	Pancolitis	8
Ileocaecal	12	Pouchitis	3
Colonic	4	Microscopic	1
Rectal	5	16 active and 9 inactive	
Perianal	4		
Microscopic	1		
20 active and 15 inactive			

TABLE 8:2 LEVELS OF SECRETORY IgA AND THE PROPORTION OF SECRETORY IgA TO THE TOTAL IgA IN WGLF OF IBD PATIENTS AND CONTROLS

GROUP	N	sIgA (ug/ml) Median (range)	%sIgA Median (range)
Control	16	151.5 (15-759)	100 (7-117)
ALL IBD	60	148.5 (30-1292)	69** (4-107)
ACT IBD	36	178 (30-1292)	67** (4-97)
INAC IBD	24	134 (39-924)	72* (40-107)

%IgA sIgA as a percentage of total IgA

*p < 0.05 levels of significance from controls

**p< 0.01 levels of significance from controls

ALL IBD = All patients with IBD

ACT IBD = Patients with active IBD

INACT IBD = Patients with inactive IBD

TABLE 8:3 LEVELS OF TOTAL WGLF IgA AND %SIgA IN ULCERATIVE COLITIS AND CROHN'S DISEASE.

GROUP	N	sIgA (ug/ml) Median (range)	%sIgA Median (range)
CONTROL	16	151.5 (15-759)	100 (7-117)
UC	25	152 (30-924)	67** (4-103)
CD	35	147 (30-1292)	71** (12-107)

%sIgA = sIgA as a percentage of total IgA

**p < 0.01 level of significance of difference from controls.

UC = ulcerative colitis

CD = Crohn's disease

TABLE 8:4 TOTAL WGLF IGA AND %SIGA IN ULCERATIVE COLITIS AND CROHN'S DISEASE ACCORDING TO DISEASE ACTIVITY.

		sIgA (ug/ml)	%sIgA
GROUP	**N**	**Median (range)**	**Median (range)**
CONTROL	16	151.5 (15-759)	100 (7-117)
aUC	16	171 (30-803)	67** (4-97)
inaUC	9	137 (50-924)	67 (10-103)
aCD	20	178 (30-1292)	68.5* (12-97)
inaCD	15	121 (39-448)	74# (45-107)

%sIgA = sIgA as a percentage of total IgA

* p < 0.05 level of significance of difference from healthy controls.

**p< 0.01 level of significance of difference from healthy controls.

p = 0.459 borderline significance

aUC = active ulcerative colitis

inaUC = inactive ulcerative colitis

aCD = active Crohn's disease

inaCD = inactive Crohn's disease.

TABLE 8:5 LEVELS OF NON-SECRETORY IgA (nsIgA) IN WGLF OF CONTROLS AND PATIENTS WITH IBD.

		nsIgA (ug/ml)	
GROUP	N	Median (range)	p
CONTROL	16	0.00 (-35-860)	
ACT IBD	36	57 (8-839)	0.0008
INACT IBD	24	42 (-9-651)	0.0169

p = probability of the difference from controls being due to chance.

TABLE 8:6 LEVELS OF NON-SECRETORY IgA (nsIgA) in WGLF IN ULCERATIVE COLITIS AND CROHN'S DISEASE ACCORDING TO DISEASE ACTIVITY.

		nsIgA (ug/ml)	
GROUP	**N**	**Median (range)**	**p**
CONTROL	16	0.00 (-35-860)	
CD	35	46 (-9-516)	0.0041
aCD	20	60 (10-516)	0.0040
inaCD	15	37 (-9-346)	0.0459
UC	25	58 (-7-839)	0.0021
aUC	16	57 (8-839)	0.0029
inaUC	9	68 (-7-651)	0.0445

p = probability of the difference from controls being due to chance.

TABLE 8:7A THE PROPORTION OF NON-SECRETORY IgA (%nsIgA) IN WGLF OF PATIENTS WITH IBD AND CONTROLS.

GROUP	N	%nsIgA Median (range)	p
CONTROL	16	0.00 (-17-93)	
ACT IBD	36	33 (3-97)	0.0031
INACT IBD	24	28 (-8-60)	0.0272

p = probability of the difference from controls being due to chance.

TABLE 8:7B THE PROPORTIONS OF NON-SECRETORY IgA %nsIgA IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS AND CROHN'S DISEASE.

GROUP	N	%nsIgA Median (range)	p
CONTROL	16	0.00 (-17-93)	
CD	35	26 (-8-88)	0.0105
aCD	20	30.5 (3-88)	0.0149
inaCD	15	26 (-8-55)	0.0528
UC	25	34 (-3-97)	0.0059
aUC	16	33 (3-97)	0.0067
inaUC	9	34 (-3-60)	0.0842

p = probability of the difference from controls being due to chance.

TABLE 8:8A COMPARISONS OF SECRETORY IgA LEVELS (sIgA) AND THE PROPORTION OF SECRETORY IgA (%sIgA) BETWEEN DISEASE GROUPS. Statistical comparisons by Mann-Whitney

	p Values	
	sIgA	%sIgA
ACT IBD vs INACT IBD	0.4416	0.2422
CD vs UC	0.6260	0.5191
aCD vs inaCD	0.4634	0.4237
aCD vs aUC	1.0000	0.7382
aUC vs inaUC	0.8208	0.6304
inaUC vs inaCD	0.4929	0.6547

p = probability of the difference being due to chance.

TABLE 8:8B COMPARISONS OF NON-SECRETORY IgA (nsIgA) AND THE PROPORTION OF NON-SECRETORY IgA (%nsIgA) BETWEEN DISEASE GROUPS. Statistical comparisons by Mann-Whitney

	p Values	
	nsIgA	%nsIgA
ACT IBD vs INACT IBD	0.1583	0.2484
CD vs UC	0.4671	0.5046
aCD vs inaCD	0.3254	0.4433
aCD vs aUC	0.5994	0.7143
aUC vs inaUC	0.5522	0.6711
inaUC vs inaCD	0.6547	0.6333

p = probability of the difference being due to chance.

CORRELATIONS

TABLE 8:9A RELATIONSHIP BETWEEN TOTAL IgA, SECRETORY IgA AND %SECRETORY IgA IN CONTROLS AND PATIENTS WITH IBD.

		Total IgA vs sIgA		Total IgA vs %sIgA	
GROUP	N	r	p	r	p
CONTROL	16	0.708	0.0001	-0.284	0.084
IBD	60	0.806	0.0001	-0.388	0.012
UC	25	0.731	0.0001	-0.231	0.039
CD	35	0.861	0.0001	-0.445	0.161

CONTROL = Controls as defined in text

IBD = all patients with inflammatory bowel disease

UC = ulcerative colitis patients

CD = Crohn's disease patients

p = probability that the correlation (r) would be a chance finding.

TABLE 8:9B RELATIONSHIP BETWEEN TOTAL IgA, SECRETORY IgA AND %SECRETORY IgA IN PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS BY DISEASE ACTIVITY.

		Total IgA vs sIgA		Total IgA vs %sIgA	
GROUP	N	r	p	r	p
aUC	16	0.690	0.0001	-0.478	0.028
inaUC	9	0.750	0.0001	0.226	0.605
aCD	20	0.797	0.0001	-0.245	0.783
inaCD	15	0.945	0.0001	-0.646	0.006

aUC = active ulcerative colitis

inaUC = inactive ulcerative colitis

aCD = active Crohn's disease

inaCD = inactive Crohn's disease.

p = probability that the correlation (r) would be a chance finding.

TABLE 8:10 RELATIONSHIP BETWEEN WGLF IgG (DISEASE ACTIVITY) AND SECRETORY IgA, %SECRETORY IgA.

		Total IgG vs sIgA		Total IgG vs %sIgA	
GROUP	N	r	p	r	p
CONTROL	16	0.182	0.415	-0.225	0.001
IBD	60	0.213	0.0001+	-0.156	0.073
ACT IBD	36	0.548	0.001	-0.218	0.201
INAC IBD	24	0.215	0.314	-0.124	0.563
UC	25	0.345	0.102	-0.044	0.666
aUC	16	0.560	0.014+	0.077	0.932
inaUC	9	0.553	0.010+	0.043	0.898
CD	35	0.143	0.001	-0.172	0.078
aCD	20	0.122	0.012	-0.251	0.185
inaCD	15	-0.060	0.446	0.135	0.631

CONTROL = Controls as defined in text

IBD = all patients with inflammatory bowel disease

UC = ulcerative colitis patients

CD = Crohn's disease patients

aUC and inaUC = active and inactive ulcerative colitis

aCD and inaCD = active and inactive Crohn's disease

p = probability that the correlation (r) would be a chance finding.

TABLE 8:11 RELATIONSHIP BETWEEN WGLF IgG (DISEASE ACTIVITY) AND NON-SECRETORY IgA, %NON-SECRETORY IgA.

		TOTAL IgG vs nsIgA		Total IgG vs %nsIgA	
GROUP	N	r	p	r	p
CONTROL	16	0.143	0.166	0.143	0.166
IBD	60	0.241	0.0001+	0.154	0.091
ACT IBD	36	0.306	0.002	0.132	0.231
INA IBD	24	-0.132	0.522	-0.117	0.558
CD	35	0.226	0.0001	0.285	0.097
aCD	20	0.436	0.0001	0.249	0.217
inaCD	15	-0.296	0.431	-0.142	0.633
UC	25	0.135	0.228	0.038	0.676
aUC	16	-0.010	0.414	-0.077	0.925
inaUC	9	0.150	0.059	0.043	0.897

CONTROL = Controls as defined in text

IBD = all patients with inflammatory bowel disease

UC = ulcerative colitis patients

CD = Crohn's disease patients

aUC and inaUC = active and inactive ulcerative colitis

aCD and inaCD = active and inactive Crohn's disease

p = probability that the correlation (r) would be a chance finding.

CHAPTER NINE:

SYSTEMIC AND SECRETORY FOOD ANTIBODIES

IN

CONTROLS AND IBD

INTRODUCTION

The hypothesis that there is an association between food antigens and inflammatory bowel disease is longstanding (see chapter 1). There have been anecdotal clinical reports of improvement when IBD patients avoided particular food antigens (Ginsberg and Albert, 1989) and also reports of elevated levels of serum antibodies to a number of food antigens (Koninckx et al.,1984; Taylor and Truelove, 1961; Lerner et al.,1989). The conclusions as to the prevalence of food antibodies in IBD have varied. Despite all this interest there is a lack of studies on mucosal immunity to food antigens in inflammatory bowel disease. Yet the main site of disease 'activity' in IBD is at the local mucosal level, quite different from the systemic (sera) compartment which in immunological respects does not mirror mucosal events.

THE AIM OF THIS STUDY

The objective of the experiments described in this chapter was to investigate humoral mucosal and humoral systemic immunity to food antigens in patients with Crohn's disease, ulcerative colitis and controls. My hypothesis was that small bowel disease was likely to show high levels of food antibodies such as occurs in coeliac disease where there is diffuse jejunal disease.

It seemed likely that antibodies to food antigens are stimulated by exposure of the damaged upper small bowel to the many antigens present in this part of the intestines.

SUBJECTS AND PROTOCOL

A total of 57 patients (38 Crohn's disease and 19 Ulcerative colitis) and 23 controls were studied (Table 9:1 in the appendix).

The macroscopic manifestations of patients at the time of lavage were as follows: orofacial (1), jejunal (1), terminal ileum (10), ileocaecal (4), colonic (9), rectal (3), perianal (2) (one associated with sigmoid-ilial fistula and the other associated with rectal involvement), microscopic colitis (1), and seven with no macroscopic disease (5 after resections). All the patients were on normal diet. As regards drug treatment 7 patients were on steroids the dose ranging between 10-40mg per day, 17 on sulphasalazine, 3 on sulphasalazine and steroids and 11 on no drug therapy for their Crohn's disease. The age range was 13-76 with a median of 38. Twenty-one had active disease and in 17 the disease was inactive as assessed clinically and also based WGLF total IgG (see chapter 7).

In the ulcerative colitis group none had had any

resections. The macroscopic distribution of the disease at time of sampling was as follows: 8 pancolitis, 4 left-sided involvement and 7 proctitis. Four were on no drug treatment, 5 were on oral prednisolone and an amino-salicylate type (ASA) drug plus rectal steroids, 3 were on an ASA group drug plus rectal steroids, 1 was on rectal steroids only, 3 on an oral ASA group only and 3 were taking oral prednisolone as well as ASA. The age range for this group was 23-81 with a median of 37. Of the 19, 12 had active disease at time of study.

Most of the controls had presented with symptoms that required gastro-intestinal investigations but whose diagnosis was non-inflammatory dysfunction. The final diagnoses in the controls were as follows: non-inflammatory polyps in the colon (5), constipation (7), diarrhoea with no abnormality detected (5), colonic angiodysplasia (1) and one each with oesophagitis, duodenal ulcer, unexplained abdominal pain and 2 healthy volunteers. The age range was 14-88 with a median of 53.

All the subjects were on normal diet

Total immunoglobulins of the IgA, IgG and IgM classes and corresponding food antibodies to gliadin (GLI), ß-lactoglobulin (BLG) and ovalbumin (OVA) were measured in serum and WGLF as described in chapter 6.

The serum was drawn on the day of gut lavage collection so that the two specimens reflected systemic and mucosal immune activity at corresponding times.

Comparisons in the levels of total immunoglobulins and antibodies to the three antigens in serum and WGLF were made between the three groups. Comparisons were also made when the disease groups were further subdivided according to whether they had active or inactive disease and according to the regional distribution of disease. Finally the correlations between total immunoglobulins and food antibodies within and between sera and gut lavage fluid were performed in order to define the relationships between the two compartments if any.

SECTION 9A

TOTAL IMMUNOGLOBULINS IN INFLAMMATORY BOWEL DISEASE PATIENTS AND CONTROLS

The most abundant immunoglobulin in serum was IgG. The levels of IgG, IgA and IgM were compared between disease groups and controls. There was no significant difference between controls and the inflammatory bowel disease patients (Table 9A:2 in the appendix). All tables are in the appendix of the chapter.

SERUM ANTIBODIES TO GLIADIN, ß-LACTOGLOBULIN AND OVALBUMIN

Serum antibodies to the three food antigens were

expressed as a percentage of a standard known to have high levels of these antibodies. The result was expressed as the figure without the percent symbol. For example a level of 10 would indicate 10% as compared to the standard.

Serum antibody levels above 10 of the standard were considered positive and the levels were considered highly positive if the were above 20% for IgA, above 40% for IgG and above 60% for IgM. Table 9A:3A shows the prevalence of high positive antibodies in the disease groups and controls. Taking antigliadin IgG, for example, 3 out of the 19 UC patients gave high positive results, 4 out of the 23 controls and 15 out of the 38 Crohn's disease patients gave high positive results. Analysis by Mann-Whitney shows that there were no significant differences between levels in patients with Crohn's disease and controls or between disease groups. This lack of significant differences applied to all the groups and antibodies (Table 9A:3B).

SERUM FOOD ANTIBODIES IN ACTIVE AND INACTIVE INFLAMMATORY BOWEL DISEASE

The patients were further subgrouped according to disease activity and the prevalence of high positive antibodies in the individual group enumerated for active and inactive UC (Table 9A:4A) and for active and inactive

Crohn's disease (Table 9A:4B).

For example of 12 patients with active ulcerative colitis (Table 9A:4A), 2 had high positive antibody levels to GLI, 3 to BLG and 2 to OVA in the IgG class. And for the 7 patients with inactive ulcerative colitis, 1 had high antibodies to GLI, 4 to BLG and 2 to OVA. The same applies to IgA and IgM class antibodies. The patients were further subdivided into subgroups according to whether they had a predominantly left sided disease (LSIDED), proctitis (PR) or pancolitis (EXTENSIVE). These were also subdivided into active and inactive disease subgroups based an clinical assessment and total WGLF IgG. The rest of Table 9A:4A is essentially the same. For example there were 7 patients with proctitis, of these two had high positive antibody levels to GLI, 4 to BLG and 2 to OVA in the IgG class.

For patients with Crohn's disease the format is the same. For example there were 24 patients with active Crohn's disease, 8 had high positive antibody levels to GLI, 6 to BLG and 8 to OVA (Table 9A:4B). The patients were further subgrouped as described earlier and their frequency of high levels of antibodies to food antigens enumerated according to disease group.

The frequency of high positive antibodies to food antigens for controls is included in both Tables 9:4A and 9:4B to allow easy comparison.

The levels of antibodies were compared between disease

groups and controls for active (Table 9A:4C) and inactive disease (9A:4D).

Statistical analysis by Mann-Whitney showed that there was no significant difference (Table 9A:4C) and this applied to all patients with active IBD and inactive IBD (Table 9A:4D).

The levels of serum food antibodies in active UC did not differ significantly from inactive UC and neither was their a significant difference between active Crohn's disease and inactive Crohn's disease (Table 9A:5).

SECTION 9B

WHOLE GUT LAVAGE FLUID STUDIES

TOTAL IGA,IGG and IGM IMMUNOGLOBULINS IN WGLF OF IBD PATIENTS AND CONTROLS

The levels of total IgG, IgA and IgM were measured in WGLF of patients with Crohn's disease and ulcerative colitis (Table 9B:1).

Controls gave a range for IgG of 1-9ug/ml with a median of 1ug/ml, IgA range of 12-478 with a median of 143 and IgM range 1-9 with a median of 3.

Crohn's disease patients had IgG range of 1-393ug/ml with a median of 21.5ug/ml, IgM range of 1-102ug/ml with a median of 9ug/ml and IgA range of 1-1217ug/ml median of 171ug/ml. The levels of IgG and IgM were highly significantly elevated compared with levels in controls ($p = 0.00001$), whereas the total IgA was not significantly different from controls (Table 9B:1).

Patients with ulcerative colitis, had a range between of total IgG of 1-20ug/ml with a median of 19ug/ml, IgM range of 2-22ug/ml with a median of 9ug/ml and IgA range of 18-551ug/ml with a median of 170.5ug/ml. The levels of total IgG and total IgM were significantly higher than controls at $p=0.00001$ and $p=0.0005$ respectively whereas the IgA was not significantly different from controls.

TOTAL IMMUNOGLOBULINS IN WGLF OF PATIENTS WITH ACTIVE AND INACTIVE INFLAMMATORY BOWEL DISEASE

Patients were subgrouped according to disease activity based on clinical assessment and whole gut lavage total IgG. All groups active and inactive Crohn's disease and active and inactive ulcerative colitis had significantly higher levels of IgM in WGLF compared with controls. The statistical comparisons gave the following highly significant results: active ($p=0.00001$) and inactive ($p=0.0037$) Crohn's disease, active ($p=0.0017$) and inactive ulcerative colitis ($p=0.0273$) [Table 9B:2A].

There was no significant difference in total IgA in WGLF between controls and disease groups regardless of disease activity (Table 9B:2B).

Disease activity was defined on the basis of total IgG levels in WGLF thus it is not surprising that active Crohn's ($p=0.00001$), and active UC ($p=0.00001$) groups had significantly higher levels of total IgG than controls. The patients with inactive Crohn's disease had total IgG values in their WGLF which were in the upper limit (1-10ug/ml) of the normal range but statistically significantly higher than the levels in controls ($p=0.0044$) while the inactive ulcerative colitis group had values in the lower range close to the controls and not significantly different from controls ($p=0.1698$).

However there was no significant difference in total IgM

or total IgA between all Crohn's patients as compared with levels in all UC patients. Even within disease groups the immunoglobulin levels for patients with active Crohn's patients' levels were not significantly different from those for patients with inactive Crohn's disease, the same applied to the patients with active and inactive ulcerative colitis (Table 9B:3).

RELATIONSHIP BETWEEN TOTAL SERUM IMMUNOGLOBULINS AND TOTAL WGLF IMMUNOGLOBULINS

The levels of total IgA, IgM and IgG in WGLF were correlated with corresponding class of total levels of immunoglobulins in serum for each disease group.
There was no strong correlation between total WGLF IgA, IgM and IgG and total serum immunoglobulins in the same classes for all the IBD patients and controls (Table 9B:4A). The IBD patients were further subgrouped into active and inactive groups based on clinical assessment and WGLF IgG and comparisons between serum and WGLF immunoglobulin of the three classes made. There was no strong correlation in these immunoglobulins between the two compartments for active disease groups (Table 9B:4B) and inactive disease groups (Table 9B:4C).

ANTIBODIES TO GLIADIN, ß-LACTOGLOBULIN AND OVALBUMIN IN WGLF OF PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS

WGLF IgG ANTIBODIES

In WGLF levels of antibodies to food antigens above 10% of the reference standard were considered positive and high. The prevalence of anti-food antibodies above this level (above 10% of standard see Chapter 6A) in Crohn's disease, ulcerative colitis and normal controls is shown in table 9B:5A. There were no positive IgG antibodies to any of the three dietary antigens tested in the controls and patients with ulcerative colitis. In the Crohn's disease group of a total of 114 measurements only 4 were positive for IgG antibodies to food antigens and since 3 of these had high levels of corresponding antibodies in serum as well, this probably reflected leaked IgG from serum. In all therefore, IgG anti-food antibodies were very low or negative in WGLF in this study. IgG anti-food antibodies were therefore not considered any further (TABLE 9B:5A).

WGLF IgM and IgA ANTIBODIES

Unlike IgG there were a few patients with positive anti-food antibodies in the IgA and IgM classes.

In patients with Crohn's disease 18 out of the 38 patients had positive antigliadin IgA antibodies as compared to only 1 out 23 (Table 9B:5A) for the control group, 15 out 38 positive IgM antigliadin compared to 1 out of 23 for the controls and there were also 15 positive IgM antiovalbumin out of 38 results in Crohn's disease patients compared to 1 out of 23 for the controls (Table 9B:5A). All these results were significantly higher than controls at $p=0.0051$, $p=0.0207$ and $p=0.0203$ respectively (Table 9B:5B).

In patients with ulcerative colitis 4 out of 19 patients had positive antigliadin IgA antibodies and 4 out of 19 had positive IgM antigliadin with only three out the 19 being positive for IgM antiovalbumin antibodies (Table 9B:5A). There was no statistical difference between these and control levels (Table 9B:5B). This lack of statistical difference from controls applied for all the food antibodies in patients with ulcerative colitis.

There was no significant difference in the levels of anti-food antibodies between ulcerative colitis and Crohn's disease.

GLIADIN, β-LACTOGLOBULIN AND OVALBUMIN ANTIBODIES IN WGLF OF CONTROLS, ACTIVE AND INACTIVE INFLAMMATORY BOWEL DISEASE

Patients were further subgrouped according to disease activity as described above.

Out of the 24 active Crohn's disease patients, 12 had positive antigliadin IgA antibodies as compared to 1 out of 23 normal controls and there were 20 out of 24 positive results for IgA antiovalbumin as compared to 13 out 23 positive among controls (Table 9B:5C). These antibody levels were significantly higher than controls at p=0.0073 and p=0.0132 respectively (Table 9B:5D).

There were 11 of 24 active Crohn's disease patients who had positive antibodies for IgM antigliadin as compared to 1 out of 23 controls and IgM antibodies to ovalbumin were positive in 10 out 24 active Crohn's disease patients and only in 1 out of 23 controls (Table 9B:5C). These antibodies were significantly higher than controls at p=0.0140 and p=0.0269 respectively. There was no significant difference in the levels of antibodies between inactive Crohn's and controls for antibodies to any of the food antigens (Table 9B:5E).

The same analysis was made for patients with active and inactive ulcerative colitis. There was no significant difference in the levels of anti-food antibodies between ulcerative colitis patients, active (Table 9B:5D) and

inactive subgroups (Table 9B:5E), and controls.

COMPARISON OF FOOD ANTIBODY LEVELS BETWEEN DISEASE GROUPS

Antibody levels to food antigens were compared between disease groups. There was no significant difference between active ulcerative colitis and active Crohn's disease for all the antibodies studied except IgA antiovalbumin which showed a borderline significance ($p=0.0441$) [Table 9B:5D]. Comparison of antibody levels between patients with inactive Crohn's and inactive UC for any of the food-antigens showed that there was no significant difference . A comparison of food antibody levels between active and inactive crohn's disease and active and inactive ulcerative colitis also showed no significant differences (Table 9B:5E and 9B:5F).

COMMENT

Despite the lack of significant differences between disease groups the antibody levels show a repeating pattern. Active Crohn's disease patients have the highest levels followed by patients with active ulcerative colitis and the inactive Crohn's group are intermediate between active ulcerative colitis and inactive ulcerative colitis. The control group has the lowest levels of antibodies.

SECTION 9C

CORRELATIONS

RELATIONSHIP BETWEEN SERUM ANTIBODY LEVELS AND LAVAGE ANTIBODY LEVELS IN CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS

Levels of antibodies to food antigens in WGLF were correlated with levels of corresponding antibodies in serum for each group of patients.

The following results showed a significant correlation: controls IgA antibodies to ß-lactoglobulin (r=0.232 p=0.00001), Crohn's IgA antibodies to gliadin (r=0.297 p=0.016) and antibodies to ß-lactoglobulin (r=0.166 p=0.001) and in ulcerative colitis patients a strong correlation for IgM antibodies to ovalbumin (r=0.464 p=0.002) [Table 9C:1].

Further analysis of these results shows that for the controls the 7 out of 23 subjects who had positive lavage IgA antibodies to ß-lactoglobulin only 1 was highly positive for serum. And for the two who had high positive IgA anti-BLG antibodies in serum only one had a positive result in WGLF (Table 9C:1; Graph 9C:1A).

For Crohn's disease of the 4 patients who had high positive IgA anti-BLG in serum 2 had positive levels in WGLF or alternatively of the 15 patients who had positive

IgA antibodies to BLG in WGLF only two were highly positive in serum (Graph 9C:1B). As for IgA antibodies to gliadin in Crohn's disease, of the 18 out of 38 patients with positive antibodies in WGLF only three had simultaneously high positive results in serum. However three of the 4 who had high positive serum IgA anti-GLI also had positive levels in WGLF (Graph 9C:1C).

For ulcerative colitis 5 patients out of 19 had high positive IgM antibodies to ovalbumin in serum and of these only 2 had positive levels in WGLF. Alternatively two of the three who had positive WGLF IgM antibodies to ovalbumin also had high positive levels in serum (Graph 9C:1D).

As the graphs 9C:1A-D show the positive correlations for these groups were accounted for mainly by negative (low) results and therefore in actual fact there is no strong correlation between positive lavage antibodies and high serum antibodies to food antigens for any of the groups. The two compartments are independent of one another.

THE RELATIONSHIP BETWEEN SERUM AND WGLF ANTIBODY OF PATIENTS WITH ACTIVE IBD

A correlation analysis between WGLF and serum antibodies to food antigens in patients with active disease (Table 9C:2) and inactive disease (Table 9C:3).

There was a strong positive correlation between serum

and lavage IgA antibodies in active Crohn's disease for gliadin (r=0.389 p=0.025) and IgM antibodies to ovalbumin in ulcerative colitis (r=0.699 p=0.0001) [Table 9C:2].
Further analysis shows that only 2 of the 12 patients out of 24 active Crohn's patients who had positive antigliadin antibodies in lavage also had high positive results in serum but the two who had high positive results in serum also had positive levels of antigliadin antibodies in lavage (Graph 9C:2A).
For the 12 patients with active ulcerative colitis, of the 3 who had high positive IgM antibodies to ovalbumin in serum, 2 had positive levels in gut lavage or alternatively of the three with positive lavage IgM anti-ovalbumin, two had high positive results in serum (Graph 9C:2B). As the graphs (9C:2A-B) show these strong correlations were also accounted for mainly by the negative results. Therefore like the other results there is no strong correlation between positive levels of WGLF and serum antibodies to food antigens for all the groups.

RELATIONSHIP BETWEEN ANTIBODIES AND TOTAL IMMUNOGLOBULINS IN WGLF OF PATIENTS WITH CROHN'S, ULCERATIVE COLITIS AND CONTROLS

Antibody levels in lavage were correlated against total levels of immunoglobulins of the same class for each group.

Total lavage IgA correlated very strongly with lavage IgA antibodies to BLG ($r=0.685$ $p=0.0001$) in patients with Crohn's disease. There was no correlation between total lavage IgA and IgA antibodies to food antigens for the controls and patients with ulcerative colitis. Total WGLF IgA in Crohn's disease patients did not correlate strongly with the levels of IgA antibodies in WGLF to gliadin and ovalbumin (Table 9C:3).

There was no strong correlation between lavage total IgM and IgM antibodies to food antigens in both ulcerative colitis and Crohn's disease (Table 9C:4). The control subjects had extremely low levels of total IgM so were not subjected to statistical analysis.

THE RELATIONSHIP BETWEEN IgA AND IgM AND ANTIBODIES IN WGLF OF PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS

Correlation analyses were done between IgA and IgM antibodies in WGLF within each group.

Only IgA and IgM lavage antibodies to ovalbumin correlated strongly with each other for Crohn's disease ($r=0.430$ $p=0.017$). However further analysis of this showed that of the 10 Crohn's disease patients out of 38 who were negative for IgA antibodies to ovalbumin in WGLF, 8 of them were also negative for lavage IgM antibodies to ovalbumin. Alternatively 28 out of the 38

Crohn's disease patients with positive lavage IgA antibodies to ovalbumin 13 were negative for IgM antiovalbumin antibodies in WGLF (Table 9C:5 and Graph 9C:5). The correlation was therefore accounted for mainly by negative values.

SECTION 9D

WGLF FOOD ANTIBODIES ACCORDING TO DISEASE ACTIVITY AND DISEASE DISTRIBUTION IN PATIENTS WITH IBD

The patient groups were regrouped according to regional involvement of disease and disease activity. The distribution and the numbers in each group are shown in table 9D:1A. My hypothesis was that patients with proximal disease involving the small bowel would have more positive results than patients with 'distal' or colonic disease.

The prevalence of positive anti-food antibodies (above 10) in disease involving different regions of the gastrointestinal tract is shown in table 9D:1B. For example there were 19 patients with UC of these 4 had positive GLI, 4 positive antibodies to BLG and 10 positive antibodies to OVA in the IgA class. Similarly the frequency of positive anti-OVA antibody levels in the IgM class was 4 for anti-GLI, 2 for anti-BLG and 3 for anti-OVA. The patients were further analysed into regional disease involvement. There were seven patients with proctitis, of these 3 had positive antibodies to GLI, 1 had positive antibodies to BLG and 3 positive antibodies to OVA in the IgA class. Similar analysis was made for patients with Crohn's disease.

The patients were further subgrouped according to

disease activity for Crohn's disease (Table 9D:1C) and for ulcerative colitis (Table 9D:1D). For example in table 9D:1D of the 7 patients who had proctitis 4 had active disease and in 3 the disease was inactive. Out of the 4 patients with active proctitis, 3 had positive levels of anti-GLI, 1 positive anti-BLG and 1 positive anti-OVA in the IgA class. For the three patients with inactive proctitis none had positive antibody levels to BLG and two had positive IgA antibodies to OVA and one was positive for IgM antigliadin.

The numbers studied in each group according to regional involvement and disease activity were too small for strong statistical analysis (Table 9D:1C and Table 9D:1D).

To further analyse the Crohn's patients the regional distribution of the disease was revised and subdivided into two broad categories; proximal involvement which including orofacial, small bowel and ileocaecal involvement; and distal including the colon and proctitis (the patient with microscopic type disease was excluded from this analysis).

The prevalence of positive levels (above 10) of anti-food antibodies in the proximal and distal groups is shown in Table 9D:2A. Though there are relatively more numbers of positive results among the proximal group, 10 positive out of 19 as compared to 7 out of 18 in the distal group for IgA antigliadin, on statistical analysis

there was no significant difference (Table 9D:2B). There was no difference either when patients were analysed according to disease activity (Table 9D:2C and Table 9D:2D).

Finally as regards total immunoglobulins in gut lavage there was no statistical difference in the levels between proximal and distal disease involvement even when patients were subdivided and corresponding active and inactive varieties were compared (Table 9D:3A, Table 9D:3B and Table 9D:3C).

GENERAL SUMMARY AND COMMENT

The measurement of total immunoglobulins by ELISA (Chapter 6A) gave actual concentration in ug/ml. Anti-food antibody levels here are expressed as a ratio to a known high standard, not as actual concentration of antibody being more related to the overall binding capacity of these antibodies. Since the same standard is used for all measurements they enable comparisons to be made by using non-parametric statistics.

The results in this study show that there is no difference in total immunoglobulins and antibodies (to the three food antigens studied) in systemic circulation between controls on one hand and Crohn's or ulcerative colitis patients on the other. And this applies for both active and inactive disease groups.

Differences only appear in WGLF with total IgM and IgG being significantly higher in the active disease groups than in controls. The antibodies to ovalbumin and gliadin are also significantly higher in active Crohn's disease than controls.

Furthermore there was no significant correlation between systemic and mucosal antibodies or immunoglobulins indicating that mucosal immune activity as measured in this study was independent of systemic immunity.

It follows therefore that if indeed there is immune dysregulation in IBD then mucosal secretions like WGLF are more likely to show the changes than serum.

Whereas WGLF IgG is mainly of systemic origin leaking into mucosal lumen (chapter 7), most of the lavage IgM is probably locally produced hence the presence of positive IgM antibodies. The lack of differences between active and inactive disease for total IgM and food antibodies implies that even during inactive disease, the immune dysregulation (upregulation) still continues, albeit at a lower level than during active disease. This pattern of raised IgM levels is found also in other immune related diseases such as coeliac disease (O'Mahony et al., 1991a) and may reflect an upregulated and persistent early immune response with dysregulation of the maturation process for the production of other classes of immunoglobulins.

The lack of correlation between the levels of total

immunoglobulins in WGLF and their corresponding anti-food antibodies may indicate that the raised antibody levels are not a result of a mitogenic (polygenic) non-specific stimulation but are instead due to specific reactions to particular food antigens.

Though Crohn's disease patients had significantly raised antibodies in WGLF compared to controls, these levels were not significantly higher than those of ulcerative colitis patients. This indicates that both in UC and Crohn's disease there is upregulated mucosal immune activity and the difference is in the degree of upregulation.

There was also no significant difference between UC and controls. This may indicate that patients with UC occupy an intermediate level in this hierarchy of upregulation between controls and Crohn's disease. Alternatively, as there are no pathognomonic criteria for their distinction (chapter 1) a few UC patients might actually be misdiagnosed and later will turn out to have Crohn's disease.

WGLF antibodies and immunoglobulins cannot be used to distinguish between proximal and distal disease. As the small bowel is the area of most intense immune activity, most of the antibodies may be of small bowel origin. However the inability in this study to show any differences between proximal and distal disease may be explained by the fact that the gut lavage technique

samples the whole gut. Perhaps if selective segmental sampling of mucosal secretions were done, differences that have not been shown could become apparent. Alternatively these result may suggest that patients with large bowel Crohn's disease may still have small bowel involvement which is not clinically apparent.

It is clear from this study that Crohn's disease patients represent a hyperimmune group as compared to controls and patients with ulcerative colitis.

If these antibodies to food antigens are involved in disease pathogenesis then the effect of treatment on their levels in lavage would be informative.

The next chapter examines the effect of the withdrawal of these food antigens by use of elemental diet on the levels of food antibodies to the three antigens and the relationship of these levels to disease remission.

APPENDIX FOR CHAPTER 9

TABLE 9:1 STUDY POPULATION

	CROHN'S DISEASE	ULCERATIVE COLITIS	CONTROLS
NUMBERS	38	19	23
M:F	16:22	6:13	8:15
AGE			
MEDIAN	38	37	53
RANGE	13-76	23-81	14-88

DISEASE DISTRIBUTION AT TIME OF LAVAGE

CROHN'S DISEASE		ULCERATIVE COLITIS	
Orofacial	1	Rectum only	7
Jejunal	1	Left sided	4
T/ileum	10	Pancolitis	8
Ilcaec	4	12 active and 7 inactive	
Colonic	9		
Rectal	3		
Perianal	2		
Microscopic	1		
No macroscopic dis	7		
21 active and 17 inactive			

TABLE 9A:2 TOTAL SERUM IMMUNOGLOBULINS (mg/ml) IN PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND IN CONTROLS

	CONTROL median (Range)	CD median (Range)	P1	UC median (Range)	P2	P3
TIgA	2.7 (1-4.1)	2.4 (0.5-6.8)	0.9203	2.04 (1.2-4.0)	0.5923	0.5205
TIgG	11.2 (9-12)	10.9 (4.6-20.4)	0.3861	10.5 (6.7-15.6)	0.3289	0.8501
TIgM	1.0 (0.4-2.7)	1.12 (0.3-2.63)	0.5594	1.1 (0.5-3.4)	0.7290	0.4445

P1 = probability that the difference between Crohn's disease and controls is due to chance.

P2 = probability that the difference between ulcerative colitis and controls is due to chance.

P1 = probability that the difference between Crohn's disease and ulcerative colitis is due to chance.

TABLE 9A:3A THE FREQUENCY OF HIGH LEVELS OF ANTIBODIES IN SERUM TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN UC, CD AND CONTROLS

		IgG			IgA			IgM		
	N	GLI	BLG	OVA	GLI	BLG	OVA	GLI	BLG	OVA
CONTROL	23	4	10	5	2	2	13	5	0	4
CD	38	15	12	13	4	4	22	13	3	3
UC	19	3	7	4	0	1	9	7	1	5

N = number of subjects in the group

TABLE 9A:3B SERUM IgA, IgG, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL	CD		UC		
	median (Range)	median (Range)	P1	median (Range)	P2	P3
aGLIA	10 (10-35)	10 (10-69)	0.2086	10 (10-14)	0.7140	0.0889
aGLIG	27 (10-60)	30 (10-86)	0.4890	19 (10-57)	0.1724	0.0602
aGLIM	37 (15-86)	46 (10-215)	0.3260	52 (10-124)	0.6334	0.3833
aBLGA	10 (10-37)	10 (21-62)	0.8234	10 (10-31)	0.3121	0.3562
aBLGG	34 (10-104)	28 (10-160)	0.5418	23 (10-83)	0.1360	0.4983
aBLGM	23 (10-49)	17 (10-152)	0.4614	21 (10-40)	0.5442	0.8061
aOVAA	22 (10-42)	25.5 (10-143)	0.5921	18 (10-66)	0.8795	0.6600
aOVAG	15 (10-75)	23 (10-290)	0.2612	23 (10-83)	0.4637	0.6721
aOVAM	38 (13-67)	29 (10-141)	0.5686	34 (10-97)	1.0000	0.4516

P1,P2 and P3 = The probability that the difference between CD, UC and controls or CD and UC respectively is due to chance.

TABLE 9A:4A THE FREQUENCY OF HIGH LEVELS OF ANTIBODIES IN SERUM TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN ULCERATIVE COLITIS IN DIFFERENT REGIONAL DISTRIBUTION (ever) AND DISEASE ACTIVITY.

		IgG			IgA			IgM		
	N	GLI	BLG	OVA	GLI	BLG	OVA	GLI	BLG	OVA
UC	**19**	**3**	**7**	**4**	**0**	**1**	**9**	**7**	**1**	**5**
aUC	12	2	3	2	0	0	6	6	1	3
inaUC	7	1	4	2	0	1	3	1	0	2
PROCTITIS	7	2	4	2	0	0	4	3	0	0
active	5	1	3	2	0	0	4	3	0	0
inactive	2	1	1	0	0	0	0	0	0	0
LSIDED	4	1	1	1	0	1	1	1	1	0
active	3	1	0	0	0	0	0	1	1	0
inactive	1	0	1	1	0	1	1	0	0	0
EXTENSIVE	8	0	2	1	0	0	4	3	0	5
active	4	0	0	0	0	0	2	2	0	3
inactive	4	0	2	1	0	0	2	1	0	2
CONTROL	**(23)**	**4**	**10**	**5**	**2**	**2**	**13**	**5**	**0**	**4**

N = The number of all the patients studied with the condition.

TABLE 9A:4B THE FREQUENCY OF HIGH LEVELS OF ANTIBODIES IN SERUM TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN CROHN'S DISEASE IN DIFFERENT REGIONAL DISTRIBUTION (ever) AND DISEASE ACTIVITY.

		IgG			IgA			IgM		
	N	GLI	BLG	OVA	GLI	BLG	OVA	GLI	BLG	OVA
CD	**38**	**15**	**12**	**13**	**4**	**4**	**22**	**13**	**3**	**3**
aCD	24	8	6	8	2	1	13	7	3	3
inaCRO	14	7	6	5	2	3	9	6	0	3
SB	11	4	3	2	2	2	3	3	0	1
active	6	1	0	0	1	0	1	2	0	0
inactive	5	3	3	2	1	2	2	1	0	1
IC	7	4	3	3	1	1	6	3	1	1
active	4	1	1	1	0	0	3	1	1	0
inactive	3	3	2	2	1	1	3	2	0	1
CN	13	4	3	5	1	0	9	4	1	2
active	9	3	2	4	1	0	5	2	1	2
inactive	4	1	1	1	0	0	4	2	0	0
MISC	7	3	3	3	0	1	4	3	1	2
active	5	3	3	3	0	1	3	2	1	1
inactive	2	0	0	0	0	0	1	1	0	1
CONTROL	**(23)**	**4**	**10**	**5**	**2**	**2**	**13**	**5**	**0**	**4**

N = The number of all the patients studied with the condition.

TABLE 9A:4C SERUM IgA, IgG, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH ACTIVE CROHN'S DISEASE, ACTIVE ULCERATIVE COLITIS AND CONTROLS EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL median (Range)	CD median (Range)	P1	UC median (Range)	P2	P3
aGLIA	10 (10-35)	10 (10-69)	0.3658	10 (10-14)	0.9861	0.3829
aGLIG	27 (10-60)	21 (10-86)	0.8149	10 (10-57)	0.4438	0.1227
aGLIM	37 (15-86)	46 (10-108)	0.4126	63 (36-124)	0.5516	0.0649
aBLGA	10 (10-37)	10 (10-27)	0.5800	10 (10-15)	0.1698	0.2829
aBLGG	34 (10-104)	18 (10-160)	0.1835	21 (10-56)	0.0499	0.3474
aBLGM	23 (10-49)	18 (10-152)	0.5301	25 (25-40)	0.6767	0.2539
aOVAA	22 (10-42)	20 (10-66)	0.8315	23 (10-57)	1.0000	0.8932
aOVAG	15 (10-75)	16 (10-204)	0.6704	22 (10-52)	0.7677	0.8273
aOVAM	38 (13-67)	29 (10-73)	0.4752	40 (10-97)	0.6894	0.2443

P1,P2 and P3 = The probability that the difference between CD, UC and controls or CD and UC respectively is due to chance.

TABLE 9A:4D SERUM IgA, IgG, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) INPATIENTS WITH INACTIVE CROHN'S DISEASE, INACTIVE ULCERATIVE COLITIS AND CONTROLS EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL median (Range)	CD median (Range)	P1	UC median (Range)	P2	P3
aGLIA	10 (10-35)	10 (10-40)	0.7661	10 (10-10)	NA	NA
aGLIG	27 (10-60)	42 (10-69)	0.0693	29 (10-43)	0.8637	0.1563
aGLIM	37 (15-86)	43 (17-215)	0.1992	44 (10-105)	0.7499	0.4334
aBLGA	10 (10-37)	10 (10-62)	0.4714	10 (10-31)	1.0000	0.7940
aBLGG	34 (10-104)	36 (13-116)	0.9376	41 (21-83)	0.8831	0.9405
aBLGM	23 (10-49)	17 (10-55)	NA	13 (10-23)	0.0735	0.2963
aOVAA	22 (10-42)	32 (10-143)	0.0685	17 (10-66)	0.7874	0.4785
aOVAG	15 (10-75)	30 (10-290)	0.4429	36 (10-83)	0.3268	0.8814
aOVAM	38 (13-67)	31 (10-141)	0.7305	31 (10-91)	0.5563	0.7091

NA = Not applicable

P1,P2 and P3 = As above

TABLE 9A:5 COMPARISONS OF SERUM ANTIBODIES BETWEEN QUIESCENT DISEASE AND ACTIVE DISEASE WITHIN DISEASE GROUPS.

	p inaCRO vs aCD	p inaUC vs aUC
aGLIA	0.5056	NA
aGLIG	0.1880	0.1083
aGLIM	0.7278	0.0759
aBLGA	0.4864	0.3525
aBLGG	0.0528	0.0546
aBLGM	1.0000	0.0584
aOVAA	0.2502	0.7998
aOVAG	0.3968	0.3749
aOVAM	0.8510	0.4990

p = probability of the difference being due to chance.

TABLE 9B:1 TOTAL WGLF IMMUNOGLOBULINS (ug/ml) IN PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS.

	CONTROL(23) median (Range)	CD(38) median (Range)	P1	UC(19) median (Range)	P2	P3
TIgA	143 (12-478)	171 (1.0-1217)	0.9937	170.5 (18-551)	0.8029	0.9850
TIgG	1 (1-9)	21.5 (1.0-393)	0.00001	19 (1-120)	0.00001	0.6908
TIgM	3 (1-9)	9 (1.0-102)	0.00001	9 (2-22)	0.0005	0.5170

P1 = probability that the difference between Crohn's disease and controls is due to chance.

P2 = probability that the difference between ulcerative colitis and controls is due to chance.

P1 = probability that the difference between Crohn's disease and ulcerative colitis is due to chance.

TABLE 9B:2A COMPARISONS OF WGLF TOTAL IgM (ug/ml) BETWEEN DISEASE GROUPS AND CONTROLS.

GROUP	N	MEDIAN	RANGE	p vs CONTROL
CONTROL	23	3	1-9	NA
aCD	24	11	1-102	0.00001
inaCRO	14	7	1-39	0.0037
aUC	12	9	3-22	0.0017
inaUC	7	9	2-19	0.0273

p = probability of the difference between the group and controls being due to chance.

TABLE 9B:2B COMPARISONS OF WGLF TOTAL IgA (ug/ml) BETWEEN DISEASE GROUPS AND CONTROLS.

GROUP	N	MEDIAN	RANGE	p vs CONTROL
CONTROL	23	143	12-478	NA
aCD	24	158	1-1217	0.7836
inaCRO	14	264	1-1109	0.6390
aUC	12	151.5	39-523	0.9584
inaUC	7	220.5	18-551	0.6864

NA = Not applicable

p = probability of the difference between the group and controls being due to chance.

TABLE 9B:2C COMPARISONS OF WGLF TOTAL IgG (ug/ml) BETWEEN DISEASE GROUPS AND CONTROLS.

GROUP	N	MEDIAN	RANGE	p vs CONTROL
CONTROL	23	1	1-9	NA
aCD	24	35	11-393	0.00001
inaCRO	14	4	1-10	0.0044
aUC	12	34	17-120	0.00001
inaUC	7	3	1-90	0.1698

p = probability of the difference between the group and controls being due to chance.

TABLE 9B:3 COMPARISONS OF WGLF TOTAL IMMUNOGLOBULINS (ug/ml) BETWEEN DISEASE GROUPS.

	p UC vs CD	p aCD(24) vs inaCRO	p aCD vs aUC	p aUC(12) vs inaUC	p inaUC vs inaCRO
LTIgA	0.9850	0.5431	0.9032	0.8883	0.7079
LTIgM	0.5170	0.2173	0.3755	0.8883	1.0000
LTIgG	0.6908	0.00001*	0.7755	0.0005*	0.2794

p = The probability that the difference would be a chance finding.

TABLE 9B:4A CORRELATIONS BETWEEN TOTAL SERUM AND LAVAGE IMMUNOGLOBULIN LEVELS IN CROHN'S DISEASE AND ULCERATIVE COLITIS.

	CROHN'S DISEASE		ULCERATIVE COLITIS	
	r	p	r	p
IgA	0.358	0.242	0.229	0.977
IgG	-0.077	0.624	-0.002	0.302
IgM	0.175	0.488	0.269	0.237

p = The probability that the correlation (r) would be a chance finding.

TABLE 9B:4B CORRELATIONS BETWEEN TOTAL SERUM AND LAVAGE IMMUNOGLOBULIN LEVELS IN ACTIVE CROHN'S DISEASE AND ACTIVE ULCERATIVE COLITIS.

	CROHN'S DISEASE		ULCERATIVE COLITIS	
	r	p	r	p
IgA	0.017	0.699	-0.297	0.568
IgG	-0.238	0.390	0.103	0.345
IgM	0.092	0.711	0.197	0.823

p = The probability that the correlation (r) would be a chance finding.

TABLE 9B:4C CORRELATIONS BETWEEN TOTAL SERUM AND LAVAGE IMMUNOGLOBULIN LEVELS IN INACTIVE CROHN'S DISEASE AND ULCERATIVE COLITIS.

	CROHN'S DISEASE		ULCERATIVE COLITIS	
	r	p	r	p
IgA	0.776	0.184	0.982	0.290
IgG	-0.063	0.675	0.632	0.985
IgM	0.224	0.591	0.982	0.542

p = The probability that the correlation (r) would be a chance finding.

TABLE 9B:5A THE FREQUENCY OF DETECTABLE ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN UC, CROHN'S AND CONTROLS.

		IgG			IgA			IgM		
	N	GLI	BLG	OVA	GLI	BLG	OVA	GLI	BLG	OVA
CONTROL	23	0	0	0	1	7	13	1	1	1
CD	38	2	0	2	18*	14	28	15*	5	15*
UC	19	0	0	0	4	4	10	4	2	3

TABLE 9B:5B WGLF IgA, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL median (Range)	CD median (Range)	P1	UC median (Range)	P2	P3
aGLIA	10 (10-30)	10 (10-82)	0.0051*	10 (10-27)	0.3902	0.0591
aGLIM	10 (10-14)	10 (10-89)	0.0207*	10 (10-14)	0.3764	0.1600
aBLGA	10 (10-22)	10 (10-121)	0.5031	10 (10-33)	0.6311	0.3058
aBLGM	10 (10-13)	10 (10-26)	0.5467	10 (10-12)	0.7617	0.8127
aOVAA	11 (10-88)	19 (10-87)	0.0521	12 (10-89)	0.8695	0.1478
aOVAM	10 (10-15)	10 (10-45)	0.0203*	12 (10-67)	0.5275	0.1526

P1 = probability that the difference between Crohn's disease and controls is a chance finding.

P2 = probability that the difference between ulcerative colitis and controls is a chance finding.

P1 = probability that the difference between Crohn's disease and ulcerative colitis is a chance finding.

TABLE 9B:5C THE FREQUENCY OF DETECTABLE ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH UC, CROHN'S DISEASE WITH REGARD TO DISEASE ACTIVITY AND CONTROLS.

	IgA			IgM		
	GLI	BLG	OVA	GLI	BLG	OVA
CD (38)	18*	14	28	15*	5	15*
aCD (24)	12*	9	20*	11*	4	10*
inaCRO (14)	6	5	8	4	1	5
UC (19)	4	4	10	4	2	3
aUC (12)	3	3	4	2	2	3
inaUC (7)	1	1	6	2	0	0
CONTROL (23)	1	7	13	1	1	1

TABLE 9B:5D WGLF IgA, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH ACTIVE CROHN'S DISEASE (24), ACTIVE ULCERATIVE COLITIS (12) AND CONTROLS (23) EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL(23) median (Range)	aCD(24) median (Range)	P1	aUC(12) median (Range)	P2	P3
aGLIA	10 (10-30)	10 (10-43)	0.0073*	10 (10-23)	0.3571	0.1109
aGLIM	10 (10-14)	10 (10-85)	0.0140*	10 (10-14)	0.5664	0.1354
aBLGA	10 (10-22)	10 (10-75)	0.5163	10 (10-33)	0.8621	0.5458
aBLGM	10 (10-13)	10 (10-26)	0.4500	10 (10-12)	0.5901	0.9065
aOVAA	11 (10-88)	20.5 (10-87)	0.0132*	10 (10-89)	0.4980	0.0541
aOVAM	10 (10-15)	10 (10-45)	0.0269*	10 (10-67)	0.3270	0.4402

P1 = probability that the difference between active Crohn's disease and controls is a chance finding.
P2 = probability that the difference between active ulcerative colitis and controls is a chance finding.
P1 = probability that the difference between active Crohn's disease and ulcerative colitis is a chance finding.

TABLE 9B:5E WGLF IgA, IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH INACTIVE CROHN'S DISEASE (inaCRO), INACTIVE ULCERATIVE COLITIS (inaUC) AND CONTROLS EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	CONTROL median (Range)	inaCRO median (Range)	P1	inaUC median (Range)	P2	P3
aGLIA	10 (10-30)	10 (10-82)	0.0541	10 (10-27)	0.7314	0.2794
aGLIM	10 (10-14)	10 (10-89)	0.2161	10 (10-11)	0.3774	0.8520
aBLGA	10 (10-22)	10 (10-121)	0.6611	10 (10-12)	0.5080	0.4118
aBLGM	10 (10-13)	10 (10-15)	0.8879	10 (10-10)	NA	NA
aOVAA	11 (10-88)	11.5 (10-80)	0.6725	18 (10-31)	0.1938	0.5506
aOVAM	10 (10-15)	10 (10-35)	0.1068	10 (10-10)	NA	NA

NA = Not applicable

P1 = probability that the difference between active Crohn's disease and controls is a chance finding.

P2 = probability that the difference between active ulcerative colitis and controls is a chance finding.

P1 = probability that the difference between active Crohn's disease and ulcerative colitis is a chance finding.

TABLE 9B:6 COMPARISONS OF FOOD ANTIBODY LEVELS IN LAVAGE IN PATIENTS WITH ACTIVE AND INACTIVE DISEASE.

	p inaCRO vs aCD	p inaUC vs aUC
aGLIA	0.7970	0.8327
aGLIM	0.4226	0.8327
aBLGA	0.9879	0.6726
aBLGM	0.6069	NA
aOVAA	0.1830	0.2204
aOVAM	0.7507	NA

NA = Not applicable

p = The probability that the difference would be a chance finding.

TABLE 9C:1 CORRELATION BETWEEN SERUM AND LAVAGE ANTIBODIES TO ß-LACTOGLOBULIN (BLG), GLIADIN (GLI) AND OVALBUMIN (OVA) IN PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AS COMPARED TO CONTROLS.

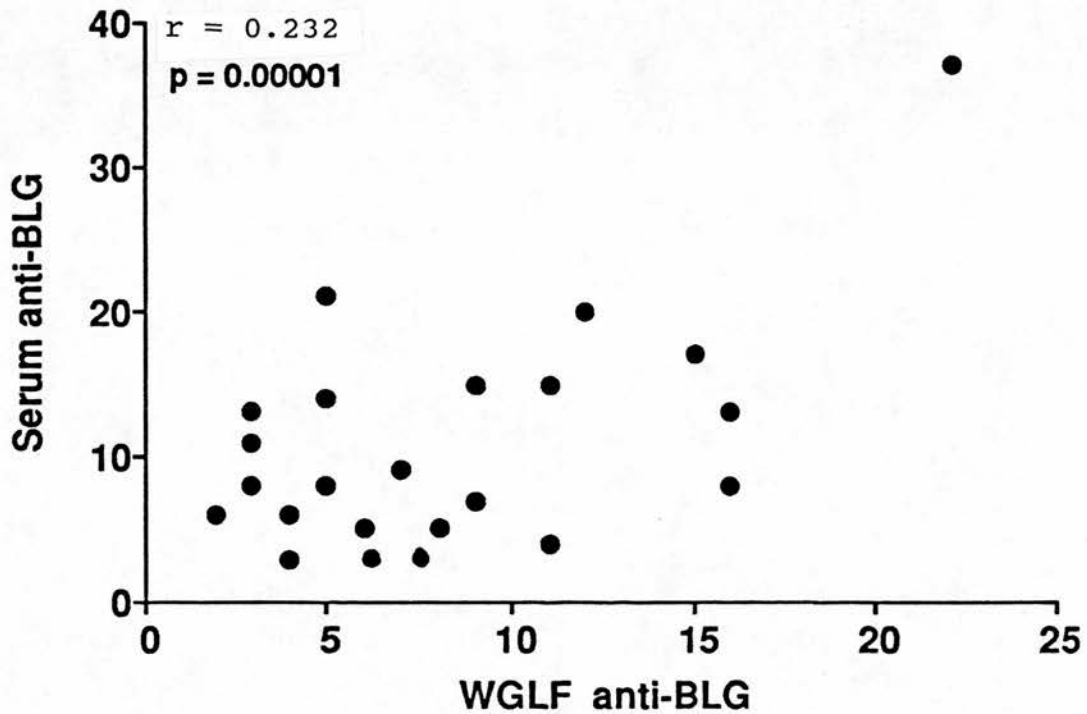
	CONTROL(n=23)		CD(n=38)		UC(n=19)	
	r	p	r	p	r	p
aGLIA	-0.066	0.826	0.297	0.016*	NA	NA
aGLIM	-0.111	0.717	0.005	0.619	-0.417	0.323
aBLGA	0.232	0.0001*	0.166	0.001*	0.392	0.937
aBLGM	NA	NA	-0.114	0.624	NA	NA
aOVAA	0.338	0.669	0.216	0.179	-0.633	0.195
aOVAM	-0.097	0.753	-0.023	0.999	0.464	0.002*

NA = Not applicable

p = The probability that the correlation (r) would be a chance finding.

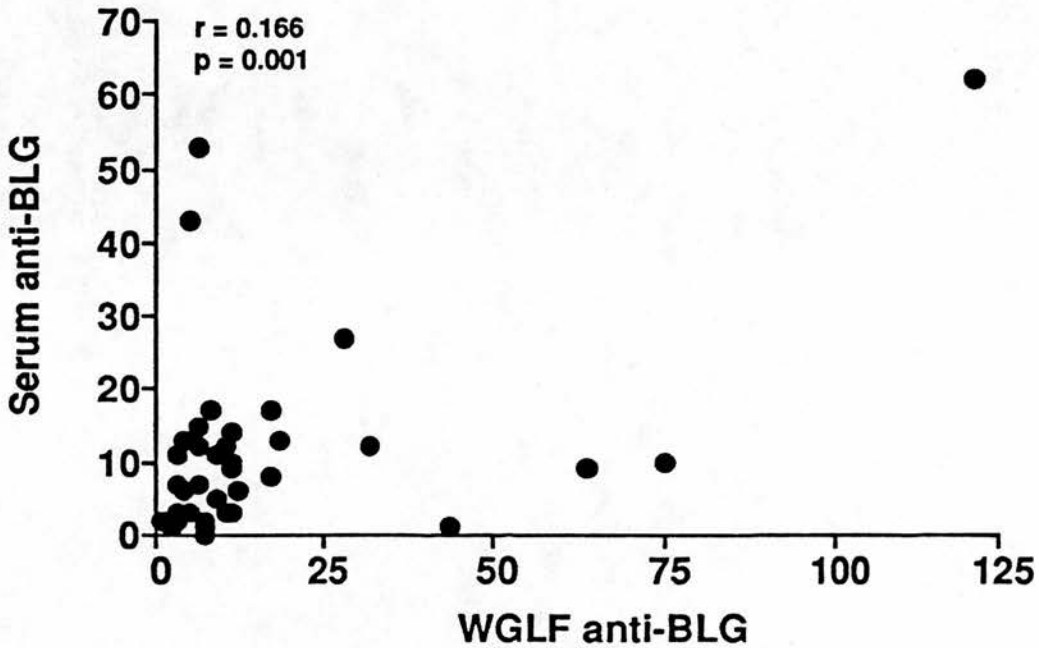
Graph 9C:1A

SERUM IgA ANTIBODIES TO BLG PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF CONTROLS



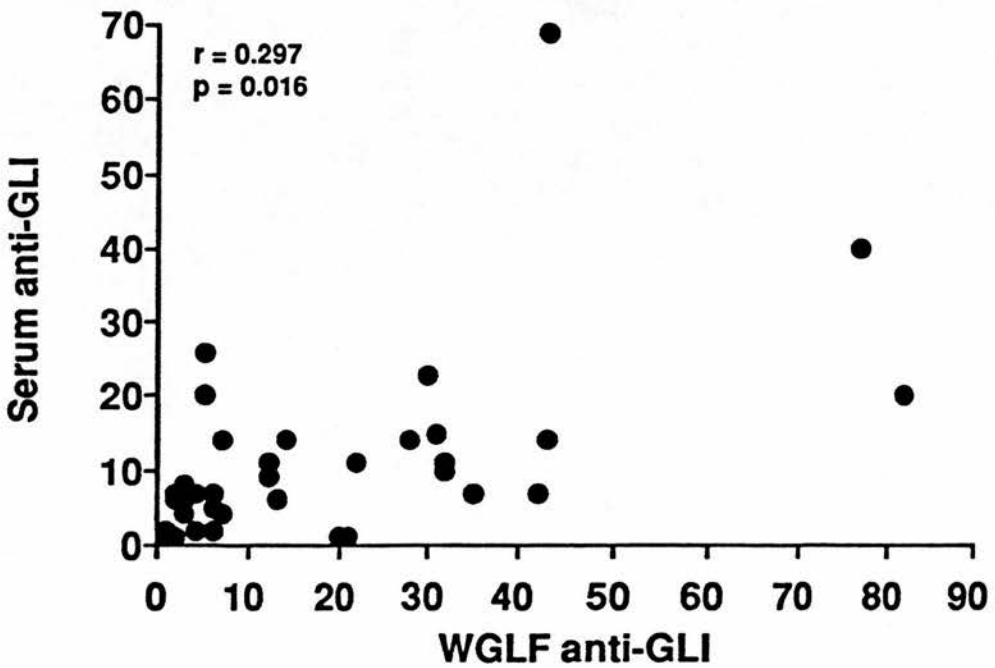
Graph 9C:1B

SERUM IgA ANTIBODIES TO BLG PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF CROHN'S DISEASE



Graph 9C:1C

SERUM IgA ANTIBODIES TO GLI PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF CROHN'S DISEASE



Graph 9C:1D

SERUM IgM ANTIBODIES TO OVA PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS

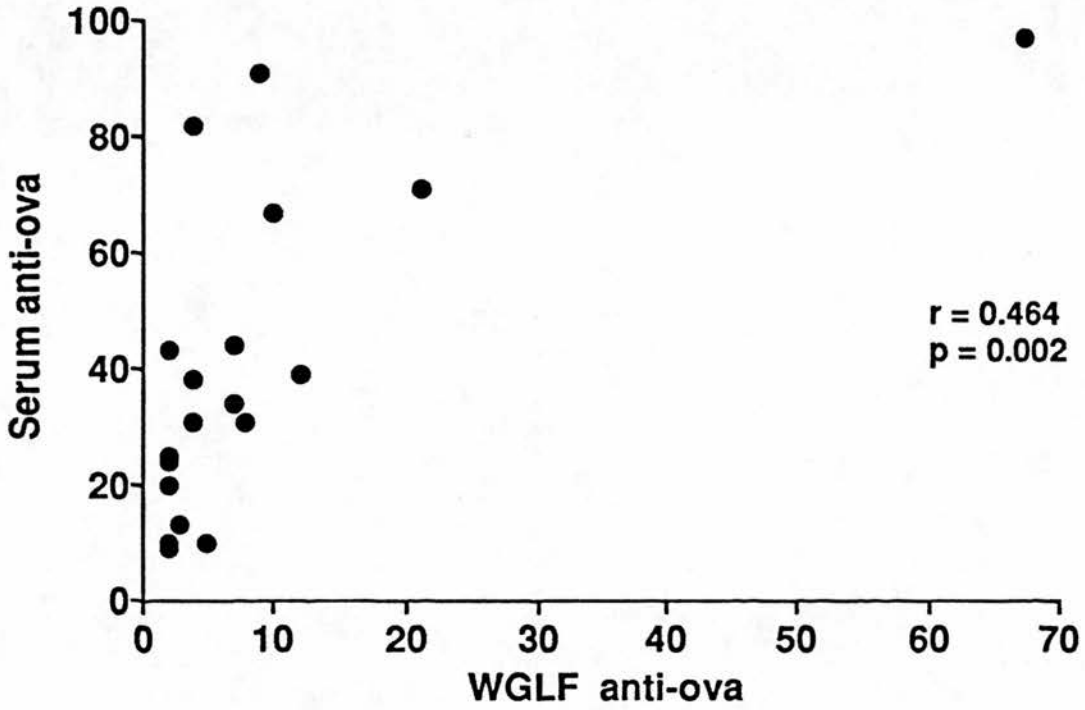


TABLE 9C:2 CORRELATIONS BETWEEN LAVAGE AND SERUM FOOD ANTIBODIES IN PATIENTS WITH ACTIVE DISEASE.

	CROHN'S(n=24)		UC(n=12)	
	r	p	r	p
aGLIA	0.389	0.025*	NA	NA
aGLIM	0.064	0.529	-0.470	0.362
aBLGA	0.295	0.450	NA	NA
aBLGM	-0.168	0.566	NA	NA
aOVAA	0.291	0.223	-0.641	0.337
aOVAM	-0.236	0.438	0.699	0.0001*

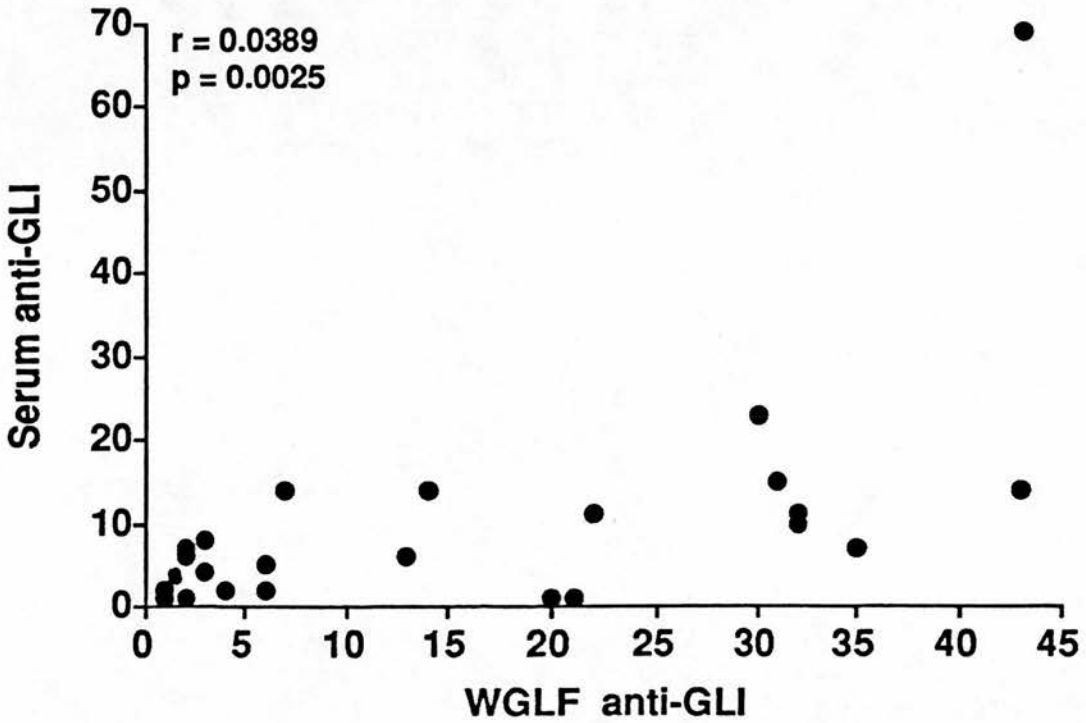
NA = Not applicable

p = The probability that the correlation (r) would be a chance finding.

The significant results were accounted for mainly by negative results (see Graphs 9C:2A-B)

Graph 9C:2A

SERUM IgA ANTIBODIES TO GLI PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE



Graph 9C:2B

SERUM IgM ANTIBODIES TO OVA PLOTTED AGAINST CORRESPONDING ANTIBODIES IN WGLF OF PATIENTS WITH ACTIVE ULCERATIVE COLITIS

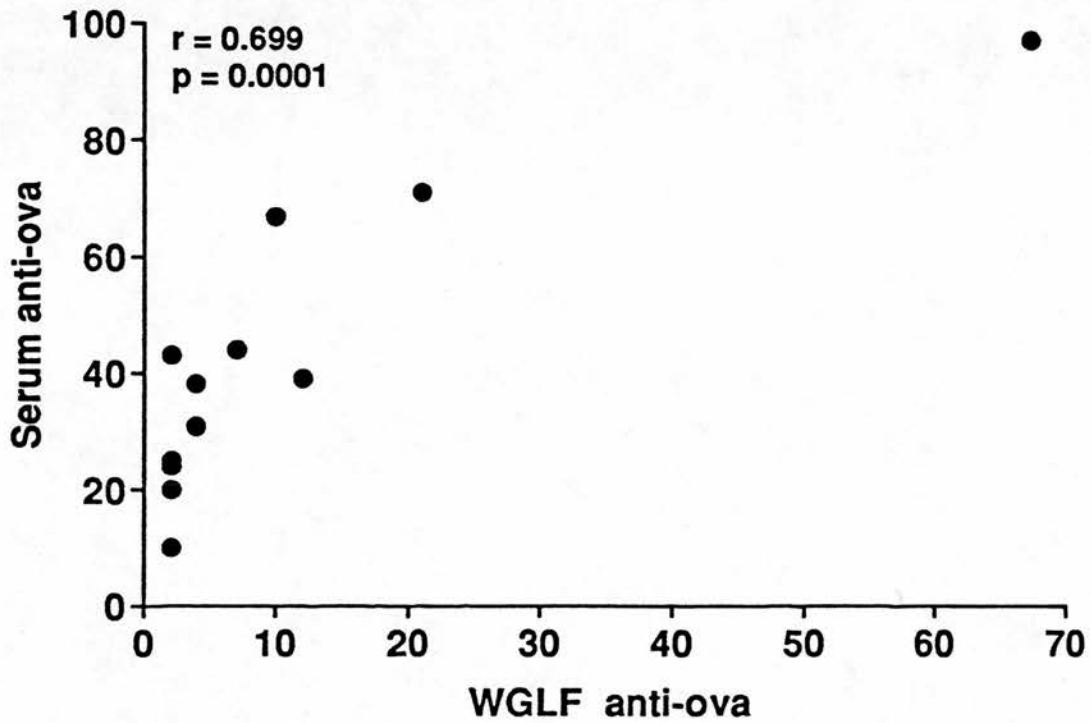


TABLE 9C:3 CORRELATIONS BETWEEN LAVAGE IgA ANTIBODIES AND LAVAGE TOTAL IgA IN CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS

	CD		UC		CONTROLS	
	r	p	r	p	r	p
BLG	0.685	0.0001*	0.416	0.088	0.167	0.456
GLI	0.376	0.057	0.207	0.410	0.402	0.065
OVA	0.333	0.472	0.012	0.264	0.169	0.452

p = The probability that the correlation (r) would be a chance finding.

TABLE 9C:4 CORRELATIONS BETWEEN LAVAGE IgM ANTIBODIES AND LAVAGE TOTAL IgM IN CROHN'S DISEASE AND ULCERATIVE COLITIS.

	CROHN'S DISEASE		ULCERATIVE COLITIS	
	r	p	r	p
BLG	0.285	0.187	0.243	0.633
GLI	0.239	0.662	0.110	0.484
OVA	0.351	0.147	0.126	0.633

p = The probability that the correlation (r) would be a chance finding.

TABLE 9C:5 CORRELATIONS BETWEEN LAVAGE IgA AND LAVAGE IgM IMMUNOGLOBULINS AND ANTIBODIES IN CROHN'S DISEASE AND ULCERATIVE COLITIS.

	CROHN'S DISEASE		ULCERATIVE COLITIS	
	r	p	r	p
LTIgA vs LTIgM	-0.170	0.679	-0.034	0.924
LGLIA vs LGLIM	0.042	0.919	0.061	0.942
LBLGA vs LBLGM	0.004	0.742	0.178	0.815
LOVAA vs LOVAM	0.430	0.017+	0.201	0.653

LTIgA = WGLF total IgA LTIgM = WGLF Total IgM

LGLIA = WGLF IgA antigliadin antibodies

LGLIM = WGLF IgM antigliadin antibodies

LBLGA = WGLF IgA antibodies to ß-lactoglobulin

LBLGM = WGLF IgM antibodies to ß-lactoglobulin

LOVAA = WGLF IgA antiovalbumin antibodies

LOVAM = WGLF IgM antiovalbumin antibodies

No significant correlation between lavage IgA and lavage IgM

+ accounted for mainly by negative values

Graph 9C:5

WGLF IgA ANTIBODIES TO OVA PLOTTED AGAINST WGLF IgM ANTIBODIES TO OVA IN PATIENTS WITH CROHN'S DISEASE

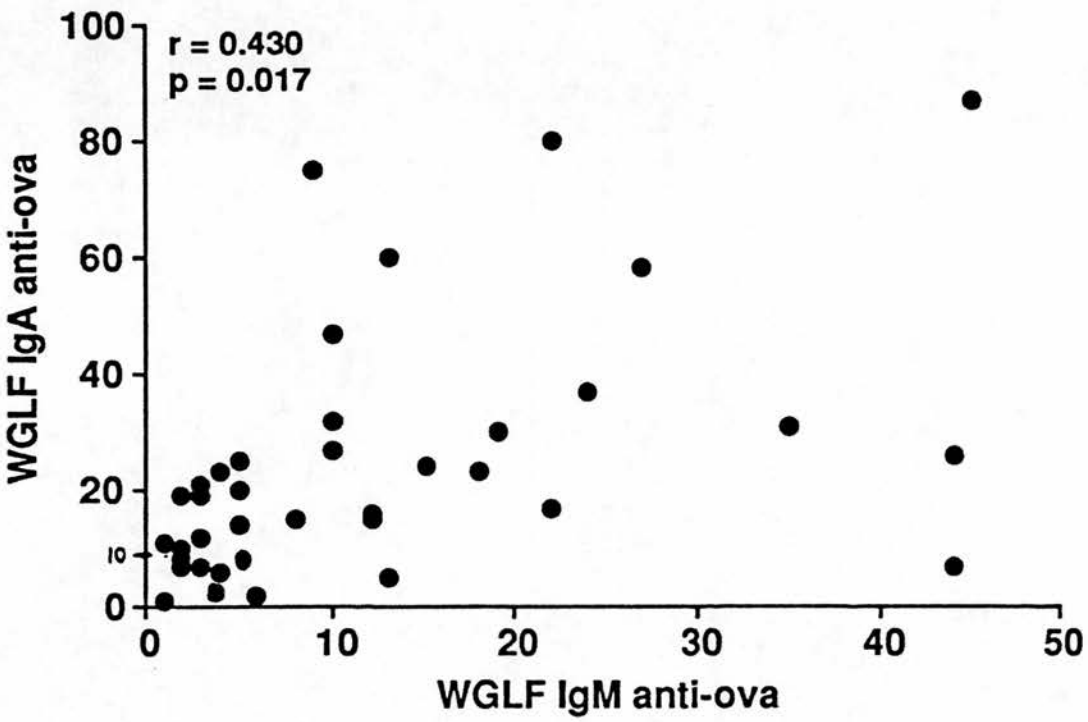


TABLE 9D:1A REGIONAL DISTRIBUTION AND DISEASE ACTIVITY GROUPS

CROHN'S DISEASE

CATEGORY		NUMBERS
ACTIVE SMALL BOWEL	(SB)	6
INACTIVE SMALL BOWEL	(SB)	5
ACTIVE ILEOCAECAL	(IC)	4
INACTIVE ILEOCAECAL	(IC)	3
ACTIVE COLONIC	(CN)	9
INACTIVE COLONIC	(CN)	4
ACTIVE MISCELLANEOUS	(MS)	5
INACTIVE MISCELLANEOUS	(MS)	2

MS - Comprised of 4 patients with Crohn's proctitis, 1 with anal involvement, 1 with orofacial and 1 with microscopic Crohn's disease.

ULCERATIVE COLITIS

CATEGORY		NUMBERS
ACTIVE PANCOLITIS	(PAN)	4
INACTIVE PANCOLITIS	(PAN)	4
ACTIVE LEFT SIDED	(LS)	3
INACTIVE LEFT SIDED	(LS)	1
ACTIVE PROCTITIS	(PR)	5
INACTIVE PROCTITIS	(PR)	2

TABLE 9D:1B THE FREQUENCY OF DETECTABLE ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN ULCERATIVE COLITIS, CROHN'S DISEASE IN DIFFERENT REGIONAL DISTRIBUTION OF DISEASE.

		IgA			IgM		
		GLI	BLG	OVA	GLI	BLG	OVA
UC (n=19)		**4**	**4**	**10**	**4**	**2**	**3**
PROCTITIS	(7)	3	1	3	1	0	0
L. SIDED	(4)	1	2	2	2	0	1
EXTENSIVE	(8)	0	5	5	1	2	2
CD (n=38)		**18**	**14**	**27**	**15**	**5**	**14**
SB	(11)	5	2	6	4	3	7
IC	(7)	4	3	6	3	0	2
CN	(13)	6	6	11	5	1	4
MISC	(7)	3	3	4	3	1	1
CONTROL(n=23)		**1**	**7**	**13**	**1**	**1**	**1**

TABLE 9D:1C THE FREQUENCY OF DETECTABLE ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN CROHN'S DISEASE IN DIFFERENT REGIONAL DISTRIBUTION AND DISEASE ACTIVITY.

		IgA			IgM		
		GLI	BLG	OVA	GLI	BLG	OVA
CD	**(38)**	**18**	**14**	**27**	**15**	**5**	**14**
aCD	(24)	12	9	19	11	4	10
inaCRO	(14)	7	5	8	4	1	4
SB	(11)	5	2	6	4	3	7
active	(6)	3	1	4	3	2	5
inactive	(5)	3	1	2	1	1	2
IC	(7)	4	3	6	3	0	2
active	(4)	2	1	4	1	0	1
inactive	(3)	2	2	2	2	0	1
CN	(13)	6	6	11	5	1	4
active	(9)	4	5	8	5	1	3
inactive	(4)	2	1	3	0	0	1
MISC	(7)	3	3	4	3	1	1
active	(5)	3	2	3	2	1	1
inactive	(2)	0	1	1	1	0	0
CONTROL	**(23)**	**1**	**7**	**13**	**1**	**1**	**1**

TABLE 9D:1D THE FREQUENCY OF DETECTABLE ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN ULCERATIVE COLITIS DISEASE IN DIFFERENT REGIONAL DISTRIBUTION AND DISEASE ACTIVITY.

		IgA			IgM		
		GLI	BLG	OVA	GLI	BLG	OVA
UC	**(19)**	**4**	**4**	**10**	**4**	**2**	**3**
aUC	(12)	3	3	4	2	2	3
inaUC	(7)	1	1	6	2	0	0
PROCTITIS	(7)	3	1	3	1	0	0
active	(4)	3	1	1	0	0	0
inactive	(3)	0	0	2	1	0	0
LEFT SIDED	(4)	1	2	2	2	0	1
active	(3)	0	1	1	1	0	1
inactive	(1)	1	1	1	1	0	0
PANCOLITIS	(8)	0	1	5	1	2	2
active	(4)	0	1	2	1	2	2
inactive	(4)	0	0	3	0	0	0
CONTROL	**(23)**	**1**	**7**	**13**	**1**	**1**	**1**

TABLE 9D:2A THE PREVALENCE OF DETECTABLE ANTI-FOOD ANTIBODIES IN PROXIMAL AND DISTAL CROHN'S DISEASE.

	IgA			IgM		
	GLI	BLG	OVA	GLI	BLG	OVA
PROXIMAL (19)	10	6	13	7	3	9
DISTAL (18)	7	8	13	7	1	4

TABLE 9D:2B IgA and IgM ANTIBODIES IN WGLF TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH PROXIMAL AND DISTAL CROHN'S DISEASE EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	PROXIMAL			DISTAL		
	MED	RANGE	N	MED	RANGE	p
LGLIA (19)	12	10-82	18	10	10-43	0.370
LGLIM (19)	10	10-85	18	10	10-89	0.9758
LBLGA (19)	10	10-121	18	10	10-75	0.5844
LBLGM (19)	10	10-26	18	10	10-21	0.6161
LOVAA (19)	19	10-60	18	18	10-87	0.7497
LOVAM (19)	10	10-44	18	10	10-45	0.2875

LGLIA and LGLIM = WGLF IgA and IgM antigliadin antibodies respectively.

LBLGA and LBLGM = WGLF IgA and IgM antibodies to ß-lactoglobulin respectively.

LOVAA and LOVAM = WGLF IgA and IgM antiovalbumin antibodies respectively.

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

TABLE 9D:2C IgA AND IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH PROXIMAL AND DISTAL ACTIVE CROHN'S DISEASE EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	PROXIMAL			DISTAL		
	MED	RANGE	N	MED	RANGE	p
LGLIA (11)	20	10-43	12	10	10-43	0.5383
LGLIM (11)	10	10-85	12	10	10-51	0.9020
LBLGA (11)	10	10-44	12	10	10-75	0.4417
LBLGM (11)	10	10-26	12	10	10-21	0.7119
LOVAA (11)	19	10-60	12	23.5	10-87	0.4060
LOVAM (11)	12	10-44	12	10	10-45	0.3248

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

TABLE 9D:2D IgA AND IgM CLASS ANTIBODIES TO GLIADIN (GLI), ß-LACTOGLOBULIN (BLG) AND OVALBUMIN (OVA) IN PATIENTS WITH PROXIMAL AND DISTAL INACTIVE CROHN'S DISEASE EXPRESSED AS A PERCENTAGE OF A KNOWN STANDARD.

	PROXIMAL			DISTAL			
	N	MED	RANGE	N	MED	RANGE	p
LGLIA	8	11	10-82	6	10	10-42	0.5186
LGLIM	8	10	10-21	6	10	10-89	0.6985
LBLGA	8	10	10-121	6	10	10-64	0.9485
LBLGM	8	10	10-15	6	10	10-10	NA
LOVAA	8	19	10-58	6	11.5	10-80	1.0000
LOVAM	8	12	10-35	6	10	10-22	0.7469

LGLIA and LGLIM = WGLF IgA and IgM antigliadin antibodies respectively.

LBLGA and LBLGM = WGLF IgA and IgM antibodies to ß-lactoglobulin respectively.

LOVAA and LOVAM = WGLF IgA and IgM antiovalbumin antibodies respectively.

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

TABLE 9D:3A TOTAL IgA and IgM IN WGLF OF PATIENTS WITH PROXIMAL AND DISTAL CROHN'S DISEASE. Statistical comparisons by Mann-Whitney.

	PROXIMAL			DISTAL			
	N	MED	RANGE	N	MED	RANGE	p
LTIgA	17	97	1-778	18	208	23-1217	0.2834
LTIgG	19	12	1-64	18	25.5	1-90	0.4384
LTIgM	17	8	1-102	18	11.5	4-59	0.3302

LTIgA = WGLF total IgA LTIgM = WGLF Total IgM

LTIgG = WGLF total IgG

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

TABLE 9D:3B TOTAL IgA, IgM IN WGLF OF PATIENTS WITH ACTIVE 'PROXIMAL' AND ACTIVE 'DISTAL' CROHN'S DISEASE. Statistical comparisons by Mann-Whitney.

	PROXIMAL			DISTAL			
	N	MED	RANGE	N	MED	RANGE	p
LTIgA	11	97	1-371	12	184	27-1217	0.4417
LTIgG	11	36	11-64	12	30.5	19-90	0.6225
LTIgM	11	8	1-102	12	12.5	4-59	0.2679

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

TABLE 9D:3C TOTAL IgA AND IgM IN PATIENTS WITH INACTIVE PROXIMAL AND INACTIVE DISTAL CROHN'S DISEASE. Statistical comparisons by Mann-Whitney.

	PROXIMAL			DISTAL			
	N	MED	RANGE	N	MED	RANGE	p
LTIgA	6	167	1-778	6	394	23-1109	0.4712
LTIgG	8	4	1-10	6	5.0	1-9	0.7963
LTIgM	6	6.5	1-39	6	7.5	4-30	0.9362

LTIgA = WGLF total IgA LTIgM = WGLF Total IgM

LTIgG = WGLF total IgG

p = probability of the difference between proximal and distal levels being a chance finding for the particular antibody.

CHAPTER TEN:

HUMORAL IMMUNITY AND ELEMENTAL DIET

INTRODUCTION

A number of workers have reported increased food antibody levels in the serum of patients with inflammatory bowel disease to specific antigens such as cow's milk protein (Taylor and Truelove, 1961) and gliadin (Koninckx et al., 1984; see chapter 2B). Crohn's disease patients have higher food antibody levels in their intestinal secretions than controls (see chapter 9).Exclusion diets have been used in inducing and prolonging disease remission in inflammatory bowel disease (see chapter 5). Elemental diets have also been shown to induce remission in acute inflammatory bowel disease (Okada et al.,1990; O'Morain et al., 1980; Giaffer et al., 1990; see chapter 5).

One postulated mechanism is that since elemental diets are devoid of polypeptides they are not antigenic. Therefore the provision of nutrition without the injurious antigenic stimulation may account for their efficacy (see chapter 5). If indeed this is the way elemental diets act then one would expect patients who respond to have positive food antibodies or sensitive T cells and a change in food antibody levels or T cell activity when on elemental diet.

AIM OF STUDY

The aim of this study was to find out whether elemental diets led to a down-regulation of humoral immune activity and whether this down regulation correlated with disease response. After studying the steady state of immunoglobulins in parotid saliva and serum the effects of elemental diet on humoral immunity were studied in four ways. The first part examined the changes in parotid saliva immunoglobulin and food antibody levels when patients are prescribed elemental diet, the second part similarly examined changes in serum immunoglobulins and food antibodies. The third part is a study of the changes in the numbers of immunoglobulin and antibody secreting cells in peripheral blood and finally changes in the levels of immunoglobulins and antibodies in whole gut lavage fluid (WGLF) were studied. The food antibodies studied were antigliadin (GLI), anti-ß-lactoglobulin (BLG) and anti-ovalbulmin (OVA) [see chapter 5]. No work so far has been done to look at the effect of elemental diets on circulatory and mucosal total immunoglobulins and antibodies to food antigens. This work aims at contributing to fill this gap.

SERUM AND SALIVA STUDY

SUBJECTS AND PROTOCOL

A total of 33 patients were studied and 31 of these had elemental diet for at least 7 days, two had a polypeptide based liquid diet, triosorbin. All the patients provided serum during the study (Table 10:1A and Table 10:1B).

Corresponding saliva and serum samples were collected from 19 patients, 13 Crohn's disease, 2 ulcerative colitis and one each with short gut syndrome, villous adenoma, carcinoma of the colon and coeliac disease. The age range was 20-82. Six patients were on steroids. In the corresponding saliva and serum study group 16 patients had elemental diet for at least 7 days.

The other 14 who provided serum specimens only comprised of 12 Crohn's disease, one each with ulcerative colitis and coeliac disease.

Saliva and serum specimens were collected before commencement of diet, at least once a week during dietary therapy and within 4-7 days after completion of the dietary regime.

For the analysis of food antibodies, only patients with highly positive initial food antibody levels as compared to a known standard (see chapter 6) were considered. Any value above 10 in parotid saliva was considered positive. Serum IgA antibody values above 20, IgG above 40 and

IgM above 60 were considered highly positive. All inflammatory bowel disease patients had active disease on global assessment when elemental diet was started.

SIMULTANEOUS SERUM AND PAROTID SALIVA STUDY

The first part of the study examines the pattern of immunoglobulins (Ig) and antibodies in saliva and serum of these patients before commencing on elemental diet. Where more than one specimen had been measured a mean value was taken.

SECTION 10A - PAROTID SALIVA STUDIES

TOTAL IMMUNOGLOBULINS IN SALIVA

IgA was the most abundant immunoglobulin in saliva, for example pre-elemental diet the range was 15-205ug/ml with a median of 90ug/ml. One patient (LD) was IgA deficient. The amounts of IgG and IgM were negligible (Table 10A:2 in appendix to this chapter). All graphs and tables for this chapter are in the appendix to this chapter.

THE RELATIONSHIP BETWEEN TOTAL SALIVA IgA AND SERUM IgA

A mean total parotid saliva IgA for each patient was calculated from all total saliva results obtained from

each patient before elemental diet. The means for serum IgA were similarly obtained.

There was no significant correlation (r=0.527, p=0.057) between mean total saliva IgA and mean total serum IgA (Graph 10A:2A in appendix to this chapter).

PATTERNS OF SALIVA TOTAL IgA LEVELS IN INDIVIDUALS

Whereas total IgA saliva levels tend to vary from day to day (Graph 10A:2B) for each individual the variation is within a very narrow range which allows for major changes which may result from dietary manipulation or drug administration to be studied.

THE RELATIONSHIP BETWEEN SMOKING AND TOTAL SALIVARY IgA

None of the patients smoked more than 20 cigarettes a day. They were grouped into three categories; group 1 smoked 0-5/day, group 2 smoked 6-10/day and group 3 11-20/day (Graph 10A:2C). There was no correlation between smoking and total salivary IgA (r=0.023, p=0.924).

THE RELATIONSHIP BETWEEN SALIVA AND SERUM FOOD ANTIBODY LEVELS

SALIVA IgG ANTIBODIES AND SERUM IgG ANTIBODIES

Eight patients had at least one positive value of IgG anti-food antibodies in saliva. One value from each of these eight patients was used to calculate correlation coefficients between the levels of food antibodies in saliva and serum.

In individuals with positive IgG anti-food antibody levels in saliva, there was a strong correlation with antibodies in serum ($r=0.364$, $p=0.045$ and Graph 10A:3A). However when the correlations were done using positivity in serum as the index there was no correlation between serum and saliva antibody levels to corresponding antigens ($r=0.233$, $p=0.804$).

There was no correlation between total serum IgG and total saliva IgG ($r=0.378$, $p=0.267$ and Graph 10A:3B). This most probably implies a leak of serum IgG antibody into the few positive IgG saliva specimens that showed a positive correlation.

THE RELATIONSHIP BETWEEN SALIVA IgA ANTIBODIES AND SERUM IgA ANTIBODIES

Saliva specimens positive for IgA food antibodies were correlated against their corresponding levels in sera. There was no significant correlation between serum IgA and saliva IgA antibodies ($r=0.307$, $p=0.146$ and Graph 10A:3C) neither was there a significant correlation between total serum IgA and total saliva IgA levels ($r=0.482$, $p=0.109$ and Graph 10A:3D).

THE EFFECT OF ELEMENTAL DIET ON TOTAL IMMUNOGLOBULIN CONCENTRATIONS IN SALIVA

Total Immunoglobulin levels in saliva were plotted for each individual at three time points; before elemental diet (Before ED), during elemental diet (During ED) and 4 or more days after elemental diet (After ED) for IgA (Graphs 10A:4A). The same was done for IgG and IgM total immunoglobulins (Graph 10A:4B).

There was no significant fall in the total salivary immunoglobulins of all classes during or after elemental diet. This applied even when patients were subdivided into responders and non-responders (Table 10A:4A, 10A:4B, 10A:4C and 10A:4D-F).

THE EFFECT OF ELEMENTAL DIET ON FOOD ANTIBODIES IN SALIVA

On global assessment 6 of the 16 patients who had parotid saliva studies improved with elemental diet. Food antibodies in the patients with initial positive levels were plotted. In parotid saliva antibody levels above 10% of the known high standard were considered positive (see chapter 6A).

Five of the 6 who improved had positive food IgA antibodies in saliva (except LM) a total of 10 positive results. And 5 of the 10 patients who did not respond also had positive food antibodies to one or more antigens a total of 9 positive results. One in this latter group (LD) was IgA deficient. Both the non IBD patients, IM (coeliac) and MD (villous adenoma), had positive initial IgA antibodies.

The positive initial food antibody levels were exclusively in the IgA class except for two positive IgM results from one patient (CC). These specimens had been contaminated with blood due to vigorous suction in the learning phase of the collection of saliva. There were no initial positive IgG antibodies.

There was no significant difference in the IgA food antibody levels before and during elemental diet. There was a statistically significant fall in IgA antibody levels after the re-introduction of a normal diet ($p=0.030$). What was more unexpected was that this

difference was accounted for mostly by those who did not improve (p=0.030) and not those who improved (p=0.477). The significance of this at 3% level is debatable in view of the many comparisons being made (Table 10A:5A, Table 10A:5B and Graph 10A:5).

SECTION 10B

SERUM TOTAL IMMUNOGLOBULINS AND ANTIBODIES IN PATIENTS ON ELEMENTAL DIET

SUBJECTS AND PROTOCOL

Serum was collected from 31 patients (including the 16 in the saliva-serum study) at commencement of elemental diet, during and 4 to 7days after elemental diet. On global assessment 10 improved (Table 10:1A and Table 10:1B previously shown).

THE EFFECT OF ELEMENTAL DIET ON TOTAL SERUM IMMUNOGLOBULINS

There was no significant fall in all immunoglobulin classes during elemental diet as compared to the values before elemental diet. There was a significant fall ($p=0.024$) in the total IgG after the diet as compared to the levels during the diet (Table 10B:1A and Table 10B:1B). This significant difference held even when only IBD patients were considered ($p=0.042$) [Table 10B:1C and Table 10B:1D].

When the subjects were subdivided into those who responded (Table 10B:1E and Table 10B:1F) and those who did not respond (Tables 10B:1G and Table 10B:1H) there

was no significant difference in all immunoglobulin levels with diet.

There were only three non IBD patients and they did not show any significant fall in immunoglobulins (Graph 10B:1J).

THE EFFECT OF ELEMENTAL DIET ON LEVELS OF ANTI-FOOD ANTIBODIES IN SERUM

The effect of elemental diet on all patients with highly positive serum antifood antibodies was studied. These were patients with antibody levels for IgA > 20%, IgG > 40% and IgM > 60% as compared to the known high standard (referred to as 'positive'). These results were plotted for three time points; before elemental diet (Before ED), during elemental diet (During ED) and 4 or more days after elemental diet (After ED). Where there was more than one highly positive result during a particular dietary regime a mean was taken.

SERUM IgA ANTIBODIES AND ELEMENTAL DIET

Five of the 10 responders had positive antibodies to at least one antigen (a total of 8 positive results), 9 of the non-responders had positive antibodies as well (a total of 12 results). Two non-IBD patients (IM and MC) had high levels of antibodies to OVA and a high level to

BLG in one (MC) and GLI in the other (IM) [Graphs 10B:2H appendix p.293].

Considering all the subjects there was no significant fall in serum levels of IgA antibodies to the three food antigens GLI, BLG and OVA while on ED the fall appearing only after patients had returned to normal diet. The difference in the levels of anti-food antibody levels between during elemental diet and after returning to elemental diet was significant (p=0.010) [Tables 10B:2A and 10B:2B].

Similarly, when only IBD patients were considered, the only significant drop in the level of anti-food antibodies was for IgA after the patients had returned to a normal diet (p=0.016) [Tables 10B:2C and 10B:2D].

However when only subjects with IBD who improved were considered there was no significant difference in anti-food antibody levels between diets (Tables 10B:2E and Table 10B:2F). Whereas those that did not improve still showed a significant fall in anti-food antibodies between during elemental diet and after returning to normal diet (p=0.022) [Tables 10B:2G and Table 10B:2H].

There were no marked changes in the levels of food antibodies in non-inflammatory bowel disease patients during and after a period of elemental diet and neither was there any change in their clinical state.

SERUM IgM ANTIBODIES AND ELEMENTAL DIET

Positive IgM antibodies to at least one antigen were found in 4 responders (a total of 5 positive results), 8 non-responders (a total of 10 results) and two non-IBD patients (a total of 3 results). Three responders showed a fall of IgM antibody levels when on elemental diet in the other two there was a rise during this period. In two of the three who showed a fall, the decline in food antibody levels continued after the re-introduction of normal diet. The specimen of serum taken after the re-introduction of normal diet for one patient (DM) was not available. The other two responders showed a rise on introduction of elemental diet which fell in one (JD) and rose further in one (PM) after the re-introduction of normal diet. The two none IBD patients referred to in the graphs as 'others' showed a downward trend but the numbers of patients involved were not adequate for statistical valuations. There was no significant fall in any of these antibodies during or after elemental diet in IBD patients (Tables 10B:2B-H and Graphs 10B:2F, p.291).

SERUM IgG ANTIBODIES AND ELEMENTAL DIET

Positive IgG antibodies to at least one food antigen were found in five responders (a total of 6 results), 12 non responders (a total of 18 results) and two non IBD

patients (a total of 4 results). There was no significant fall in the antibody levels during and after a period of ED regardless of the clinical response of IBD patients (Tables 10B:2B-H as above and Graphs 10B:2G, p.292)

SECTION 10C

THE NUMBERS OF IMMUNOGLOBULIN AND ANTIBODY SECRETING B CELLS IN PERIPHERAL BLOOD BEFORE AND DURING ELEMENTAL DIET

INTRODUCTION

The AIM of this phase of the study was to find out whether there was a fall in antibody activity attributable to treatment with ED which was not detectable by the less sensitive ordinary ELISA but would be detected by an ELISPOT assay (Described in chapter 6B).

SUBJECTS AND PROTOCOL

A total of nine patients were studied (Table 10C:1A). All the IBD patients had active disease on global assessment. Eight had inflammatory bowel disease and one had coeliac disease. The age range was 23-34. All the patients consumed an elemental diet for at least 7 days, and the clinical condition of three improved.

Venous blood was drawn before, and on the seventh or later days of elemental diet. The lymphocytes were separated and prepared for ELISPOT as described in chapter 6B.

TOTAL IMMUNOGLOBULIN SECRETING CELLS AND ELEMENTAL DIET

There were no significant changes in the numbers of peripheral blood B cells secreting IgA, IgG and IgM during the diet compared to initial numbers (Graph 10C:1A; Tables 10C:1B and Table 10C:1C).

ANTI-FOOD ANTIBODY-SECRETING CELLS AND ELEMENTAL DIET

There were few class-specific antibody producing cells to any of the three dietary antigens (OVA, BLG, GLI) [Graph 10C:2A]. For all patients there were more IgA antibody secreting B-cells (range 5-180 per million lymphocytes), few IgG and IgM antibody secreting cells (ranges 4-19 per million and 3-10 per million, respectively) [Data not shown].

There was no significant fall in the number of IgM, IgG and IgA antibody secreting cells attributable to ED when all the patients were considered. However when only patients with IBD were considered there was a borderline significant fall in the numbers of IgA antibody spots during elemental diet ($p=0.043$) [Graph 10C:2A and Table 10C:2A].

COMMENT

These results show that the ELISPOT assay did not detect any reduction in antibody or immunoglobulin secretory activity due to ED. These results confirm those obtained in chapter 10A and 10B with ordinary ELISA for saliva and serum respectively. Even here the significant fall in IgA secretory activity of 1:20 ($p=0.043$; Table 10C:2A) is debatable in view of the number of comparisons being made.

SECTION 10D

WHOLE GUT LAVAGE (WGLF) IMMUNOGLOBULIN AND FOOD ANTIBODIES IN PATIENTS ON ELEMENTAL DIET

INTRODUCTION

Systemic immunity does not necessarily reflect mucosal immunity, as changes at the mucosal level may not be reflected in peripheral circulation (this has been discussed in chapters 2 and 9).

The aim of this phase of the study therefore was to find out whether there was a fall in the levels of food antibodies in gut lavage fluid when patients were on elemental diet, as it was in this compartment that higher levels of anti-food antibodies in IBD patients compared to controls were found (see chapter 9).

SUBJECTS AND PROTOCOL

Lavage specimens were collected prior to commencing and after more than 7 days of dietary treatment from 12 patients with inflammatory bowel disease (Table 10D:1A). Eleven patients had elemental 028, and one (FK) had a polypeptide based diet (Triosorbin). One of the elemental diet patients had ulcerative colitis, the rest had Crohn's disease. All IBD patients had active disease and

9 of them met the lavage IgG criteria for active disease (total lavage IgG >10). Three of these patients showed clinical improvement (DM, DB, BS). One non-IBD (Coeliac) patient was also studied. One patient DM was IgA deficient.

TOTAL WGLF IMMUNOGLOBULINS AND ELEMENTAL DIET

Seven of the patients showed a rise in total WGLF IgA immunoglobulins during ED, three showed a fall and in one was IgA deficient. Nine showed a fall in total WGLF IgG, one showed a rise (SJ) and in two the levels remained unchanged (MI,BS). Total WGLF IgM fell in five patients when on elemental diet, rose in five patients and in one (DS) they levels remained unchanged (Graph 10D:1A).

Analysis of these levels showed that was no significant difference in the levels of IgA and IgM immunoglobulins before ED and during ED. There was a fall in the levels of IgG of borderline significance (p=0.044) [Table 10D:1C).

THE EFFECT OF ELEMENTAL DIET ON WGLF FOOD ANTIBODIES

Positive food antibodies were mainly in the IgA and IgM class. Of the eleven IBD patients on elemental diet 6

had positive IgA antibodies to one or more of the three dietary antigens (a total of 15 results). There were four positive IgG antibody results, 3 from one patient (CCamp) and one from patient AM. The IgG was probably derived from serum (see discussion of WGLF IgG and disease activity in chapter 7). Seven of the eleven IBD patients had positive IgM food antibodies to one or more antigens (A total of 13 positive IgM antibody results).

There was no significant difference in the food antibody levels for the IgA and IgM class between before and during elemental diet. There was a fall in IgG antibodies ($p=0.001$) but this was for the two patients with a total of four positive results (Table 10D:2A and Table 10D:2B). Both these patients did not improve after the diet. The individual results are plotted in Graph 10D:2A.

The patient (FK) on the polypeptide diet had initial positive IgM and IgA anti-ovalbumin. The IgA antibody levels fell and the IgM antibody levels rose while on the diet (Graph 10D:2A). The clinical condition of this patient improved. The coeliac patient (ES) had positive IgA and IgM antibodies to all three antigens before beginning ED which fell when on elemental diet and her condition did not change (Graph 10D:2B).

GENERAL COMMENTS

SALIVA and SERUM IMMUNOGLOBULINS AND FOOD ANTIBODIES

In saliva the most abundant immunoglobulin class is IgA as would be expected, the levels of IgG and IgM are very low and the saliva samples that had levels above 10ug/ml had probably been contaminated with blood.

There was no significant correlation between saliva and blood total IgA. This confirms what is already known that the two compartments are independent (O'Mahony et al., 1991a).

The lack of correlation between the levels of IgA specific for dietary antigens in serum and saliva also adds further evidence to the independence of the two compartments as already reported (discussed in chapter 2B and chapter 9). Whereas there are day to day fluctuations in the levels of salivary IgA immunoglobulins, each individual tends to have their levels within a narrow range so that it is possible to study large fluctuations caused by disease or treatment.

The absence of correlation between smoking and total lavage IgA in saliva in this study may be due to the fact that most of this patient group were non-smokers and of those who smoked none smoked more than twenty a day. Smoking would not influence the results in this case as only those who smoked more than 20 per day had lower

levels of total parotid saliva IgA (Barton et al ., 1990). There were no significant falls in salivary and serum immunoglobulins or antibodies during elemental diet regardless of the patient group or the outcome. The paradoxical significant falls in saliva IgA antibodies occurred only after the re-introduction of normal diet as compared to the levels during ED (p=0.031). When this is further analysed the significant fall was seen only in non-improvers (p=0.030) and not those who improved (p=0.477) [Table 10A:5B]. Total serum IgG fell after the re-introduction of normal diet (p=0.024; Table 10B:1B). However when the patients were divided into responders and non responders, there was no significant difference (Table 10B:1F and 10B:1H). Serum IgA antibodies also fell when patients were back on normal diet(p=0.016, Table 10B:2D), the fall being confined only to the non-responders (p=0.022; Table 10B:2H) and not responders (p=0.234; Table 10B:2F). This could be a delayed detection of the effect of the diet but then one would have expected the fall in antibodies to be confined to the patients who improved. Furthermore the numbers of antibody secreting cells as measured by ELISPOT, which would show changes earlier (see chapter 6C), do not support a possible early difference that may have not been detected by ordinary ELISA. I propose that this is a chance finding.

IMMUNOGLOBULIN AND ANTIBODY SECRETING CELLS IN THE PERIPHERAL BLOOD OF PATIENTS ON ELEMENTAL DIET

There were more B cells secreting IgA than the other two classes. This confirms similar findings using other techniques (Kutteh et al., 1980).

There were no changes in the numbers of class-specific immunoglobulin producing cells before as compared to the numbers during ED. The significant fall in IgA anti-food antibody secreting cells ($p=0.043$; Table 10C:2A) is influenced unduly by one result (BS). Without this result the changes in the number of IgA antibody secreting cells due to ED become non significant ($p=0.075$).

WHOLE GUT LAVAGE IMMUNOGLOBULINS AND FOOD ANTIBODIES IN PATIENTS ON ELEMENTAL DIET

There was no fall in total IgA or IgM when patients were on elemental diet. The borderline significant fall ($p=0.044$, Table 10D:1C) in total WGLF IgG which is a measure of disease activity in inflammatory bowel disease (see chapter 5) would indicate reduced disease activity in the group as a whole despite the individual variations. As before this significance of 1:20 is debatable in view of the many comparisons being made. The IgG immunoglobulin is probably plasma derived, and the fall in WGLF total IgG indicates reduced leakage from

plasma. Similarly the significant fall in IgG antibodies ($p=0.001$) involves only two patients and this class of antibodies are most likely serum derived (see chapter 9), this fall therefore does not reflect a specific effect of the diet on mucosal immunity.

SUMMARY

The expected response rate to elemental diet in acute inflammatory bowel disease is about 80%, equivalent to the response to steroid therapy (O'Morain et al., 1984; Okada et al., 1990). The proportion of responders is much lower in this study, about 30%. Invariably the patients in this study were prescribed elemental diet only when other treatments had failed to alleviate their clinical disease. Elemental diet was by no means a first line treatment nor was the study evaluating the efficacy of elemental diet.

The consistent feature of this study was that there was no fall in antibody levels in serum, saliva and whole gut lavage fluid when patients were on an elemental diet i.e a diet which is devoid of antigenic polypeptides. There was no fall either in the number of antibody secreting cells in peripheral blood reflecting that a delayed detection in the serum could not account for the lack of significant differences in serum ELISA results. This work therefore suggests that down regulation of

systemic and mucosal B cell antibody activity IS NOT the mechanism of action of elemental diet.

Though only one coeliac patient had WGLF studies the dramatic fall in the antibody levels for food antigens needs to be pursued with a study of more cases.

TABLE 10:1A

SERUM AND PAROTID SALIVA ANTIBODIES AND ELEMENTAL DIET

						CLINICAL DATA															
NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	TP	ALB	HB	CRP	ESR	WT	TP	ALB		CO		
JD	44	M	1COLI	ACUTE EXARCERBATION	NONE	9	12	11	108	74	65	33	13	6.5	60	71	66	33		*		
FF	27	F	1	SUBACUTE OBSTRUCTION	NONE	8	14	1.5	12	64	57	30	15	1.5	5	65	73	43		*		
TK	22	M	1COLI	STEROID FAILURE	NONE	12	12	10	58	59	65	30	11	1.5	18	60	70	30		*		
CCr	45	F	1	ACUTE EXACERBATION	COL+ILEUM	9	15	12	50	51	70	39	15	1.5	72	55	81	44		*		
PM	23	F	1	ACUTE EXACERBATION	NONE	7	13	1.5	36	52	56	27	11	1.5	32	53	64	31		*		
LM	37	F	1ILCI	ACUTE EXACERBATION	NONE	15	9.2	6.9	68	52	65	34	10	1.5	45	55	65	35		*		
JR	44	M	1COLI	STRICTURES	R. HEMICOLON	13	13	1.5	8	55	62	32	12	1.5	8	59	70	38		D		
LD	28	F	1	ACUTE EXACERBATION	TERMINAL ILEUM	14	12	1.5	16	42	52	29	12	7.3	22	43	53	30		D		
JP	26	M	1	BURST ABD. ABSCESS	NONE	14	11	2.1	76	62	72	38	10	3.8	40	61	60	32		D		
AB	90	F	1	TO IMPROVE NUTRITION	NONE	7	10	1.5	67	–	61	32	10	1.5	72	–	53	26		D		
FH	65	M	1	MULTIPLE ILCOL STRICTURES	T/ILEUM+R COLON	12	11	1.5	30	63	62	34	12	1.5	10	64	60	32		D		
DS	30	F	1ILCI	RECURRENT ACTIVE DISEASE	RIGHT COLON	31	10	3.6	25	42	51	30	11	3.7	32	44	59	31		D		
ME	82	F	2	WEIGHT LOSS	NONE	14	10	5.2	70	–	57	32	10	3.0	55	–	68	38		D		
DSi	30	M	2	ACUTE EXACERBATION	TOTAL COLECTOMY	10	9.8	10	21	63	58	–	–	5.5	30	60	–	–		D		
MC	45	F	–	SHORT GUT SYNDRO.	ILEO-JEJUNAL	8	12	–	5	55	67	37	13	–	5	56	68	36		N		
IM	37	F	3	NOT RESPONDING TO GLUTEN FREE DIET	NONE	21	9.2	6.9	68	52	56	31	10	1.5	4.5	55	62	35		N		
ABR	87	M	–	CA COLON OBSTRUCTION	NONE	10	9.2	–	80	64	–	–	12	–	75	64	–	–		N		
MD	52	F	–	VILLOUS ADENOMA – DIARRHOEA	NONE	8	14	1.5	8	70	73	–	13	1.5	8	72	83	–		N		
FK	44	F	1	ACUTE EXACERBATION	RIGHT COLON	14	9.5	15	25	41	66	38	11.5	1.5	10	41	66	44		D		

*←BEFORE ELEMENTAL DIET→ **ON ELEMENTAL DIET→ *

ABBREVIATIONS

1 = ILEAL CROHN'S DISEASE
COL = COLONIC
ILC = ILEOCOLONIC

2 = ULCERATIVE COLITIS
3 = COELIAC DISEASE

CLINICAL OUTCOME (CO)

* = IMPROVED
D = DETERIORATED
N = NO CHANGE
DOD = DAYS ON DIET

TABLE 10:1B

SERUM ANTIBODIES ONLY AND ELEMENTAL DIET

						CLIINICAL DATA															
NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	TP	ALB	HB	CRP	ESR	WT	TP	ALB	CO			
DM	29	F	1	STEROID FAILURE	NONE	21	13	2.3	37	50	71	36	13	1.5	15	49	71	38	*			
DD	28	F	1	ACUTE EXACERBATION	ILEUM + RCOLON	9	11	6.6	21	–	54	28	11	1.5	13	–	54	27	*			
MA	34	F	1	DIARRHOEA	NONE	10	15	–	1	62	67	40	15	–	1	62	71	42	D			
CH	70	F	1	ACUTE EXACERBATION	ILEUM + CAECUM	9	12	10	96	56	72	35	11	15	102	56	67	31	D			
DC	24	M	1ILC	ACUTE EXACERBATION	ILEOJEJUNAL	21	16	3.1	14	–	71	35	17	1.5	3	–	68	34	D			
DB	19	M	1ILC	ACTIVE DISEASE	ILEUM +CAECUM	21	11	5.5	63	52	79	36	10	1.5	37	52	76	34	*			
CCam	16	M	1COU	ACTIVE CROHN'S DISEASE	STOTAL COLECTOMY	14	10	7	25	24	74	28	15	1.5	33	25	78	31	D			
JM	54	F	1COU	RECURRENT ACTIVE DISEASE	SIGMOID	14	10	10	46	66	65	32	10	1.5	42	64	66	33	D			
SJ	25	M	1COU	ABDOM. PAIN AND DIARRHOEA	NONE	21	14	1.5	33	82	82	40	14	1.5	13	83	78	40	D			
EW	25	F	1COU	RECURRENT CROHN'S DISEASE	NONE	14	11	2.0	83	45	64	34	10	3.9	80	45	64	34	D			
MI	50	F	1	RECURRENT ACTIVE DISEASE	RT. COLON	21	14	1.5	18	–	64	31	13	1.5	16	–	57	30	D			
AM	37	F	1	ACUTE EXACERBATION	TOTAL COLECTOMY	11	13	3.6	34	68	69	36	15	1.5	17	68	76	42	D			
BS	28	M	2	STEROID FAILURE	NONE	10	11	10	90	46	64	34	11	1.5	50	51	64	34	*			
ES	34	F	3	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	80	43	12	–	7	40	75	43	N			

"← BEFORE ELEMENTAL DIET →" "← ON ELEMENTAL DIET →"

ABBREVIATIONS

1 = ILEAL CROHN'S DISEASE
COL = COLONIC
ILC = ILEOCOLONIC
2 = ULCERATIVE COLITIS
3 = COELIAC DISEASE

CLINICAL OUTCOME (CO)

* = IMPROVED
D = DETERIORATED
N = NO CHANGE

DOD + DAYS ON DIET

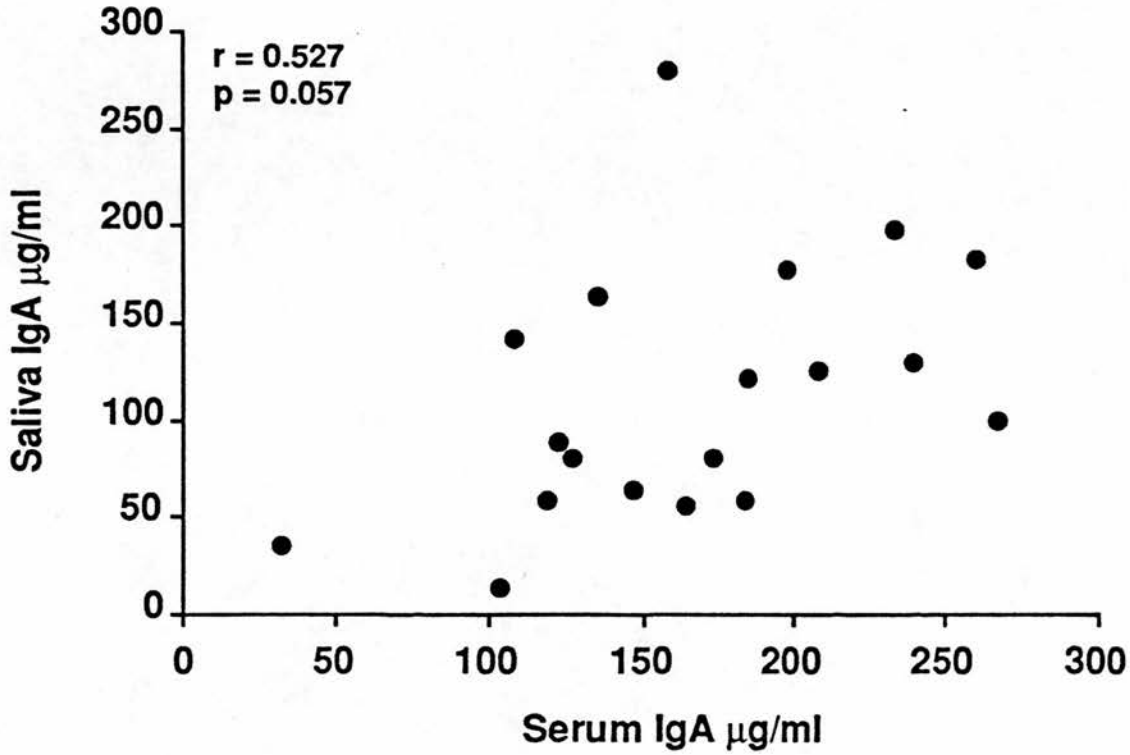
APPENDIX FOR CHAPTER 10

TABLE 10A:2 TOTAL SALIVA IMMUNOGLOBULINS (ug/ml) BEFORE ELEMENTAL DIET.

	N	MED	RANGE
IgA	15	90	15-205
IgG	16	1.5	1-19
IgM	16	1.5	1-8

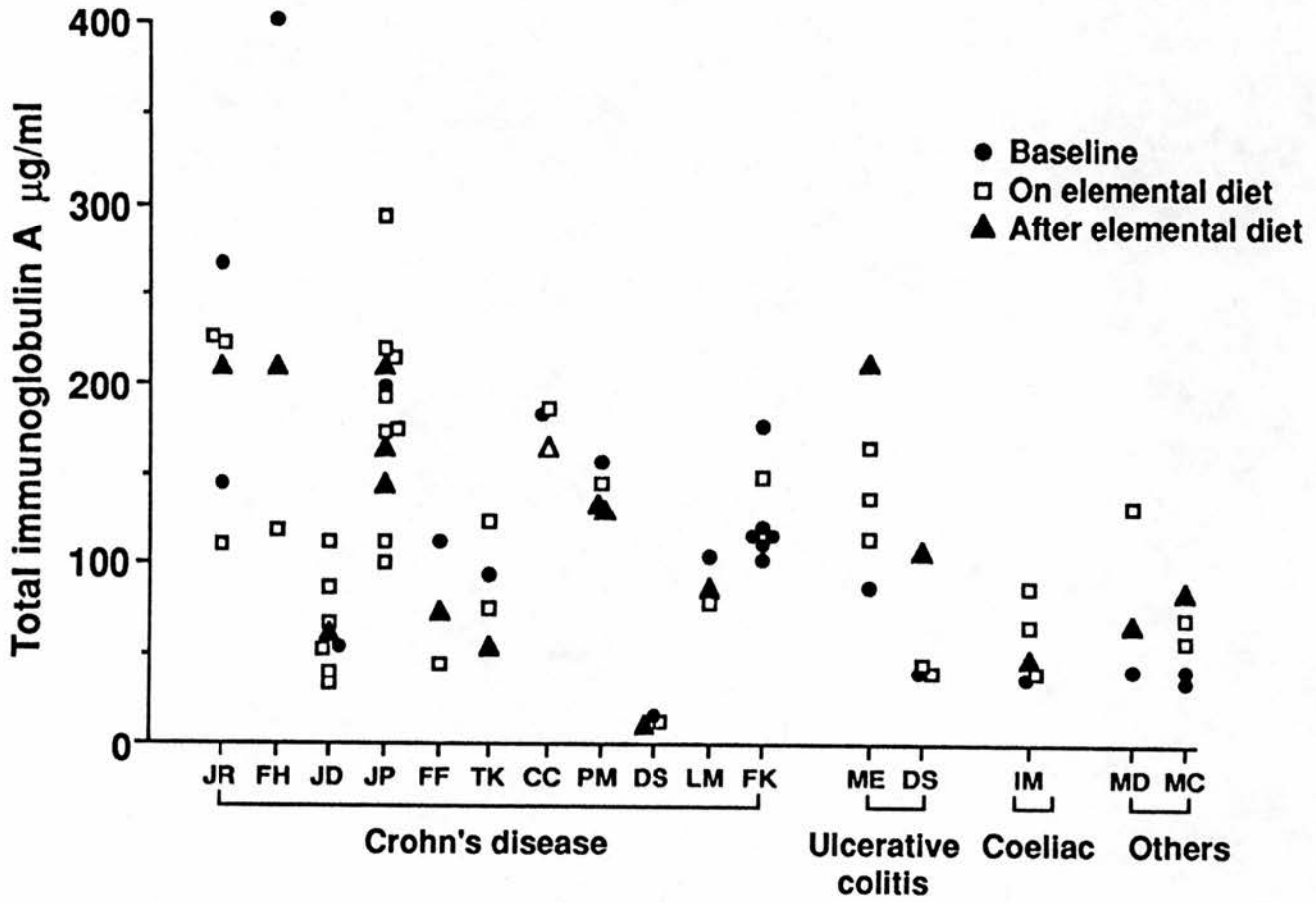
Graph 10A:2A

MEAN TOTAL SALIVARY IgA PLOTTED AGAINST MEAN TOTAL SERUM IgA



Graph 10A:2B

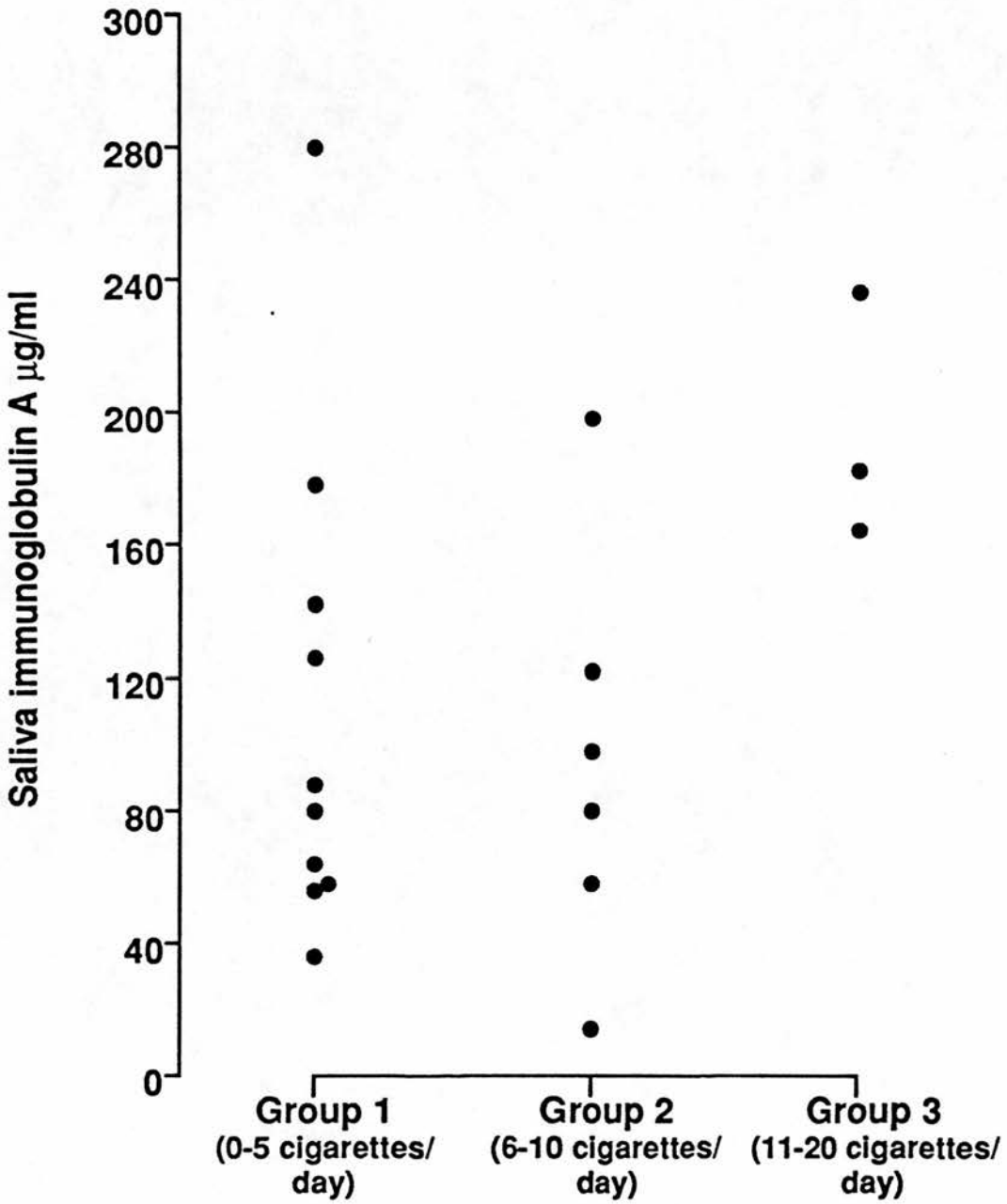
THE PATTERN OF PAROTID SALIVA TOTAL IgA IN INDIVIDUAL PATIENTS



Parotid saliva IgA remained within a narrow range for each subject.

Graph 10A:2C

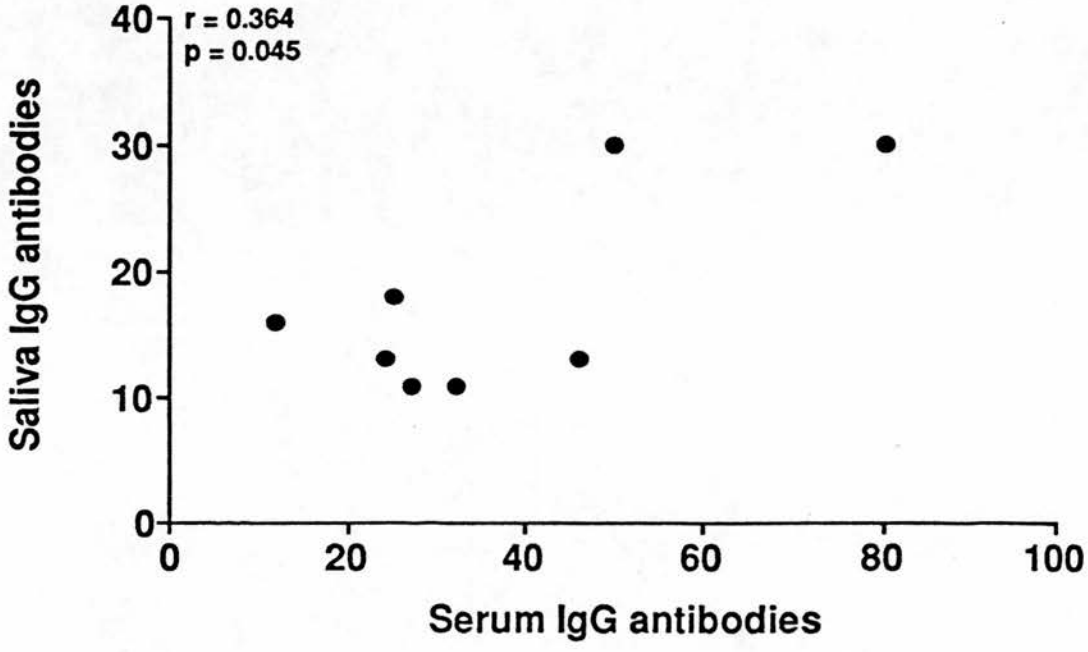
TOTAL PAROTID SALIVA IgA LEVELS IN RELATION TO CIGARETTES SMOKED



On the x axis patients were divided into three groups according to numbers of cigarettes smoked per day. On the y axis are the concentrations of IgA. There was no difference between groups.

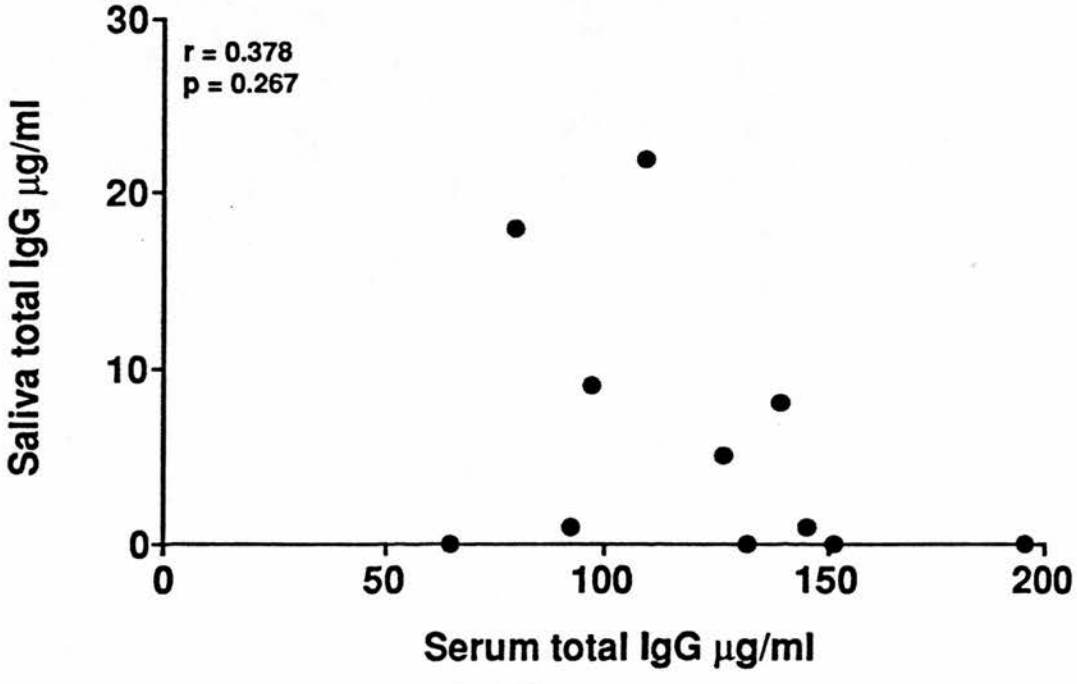
Graph 10A:3A

PAROTID SALIVA IgG ANTIBODIES PLOTTED AGAINST CORRESPONDING ANTIBODIES IN SERUM OF PATIENTS WITH POSITIVE SALIVA IgG ANTIBODIES



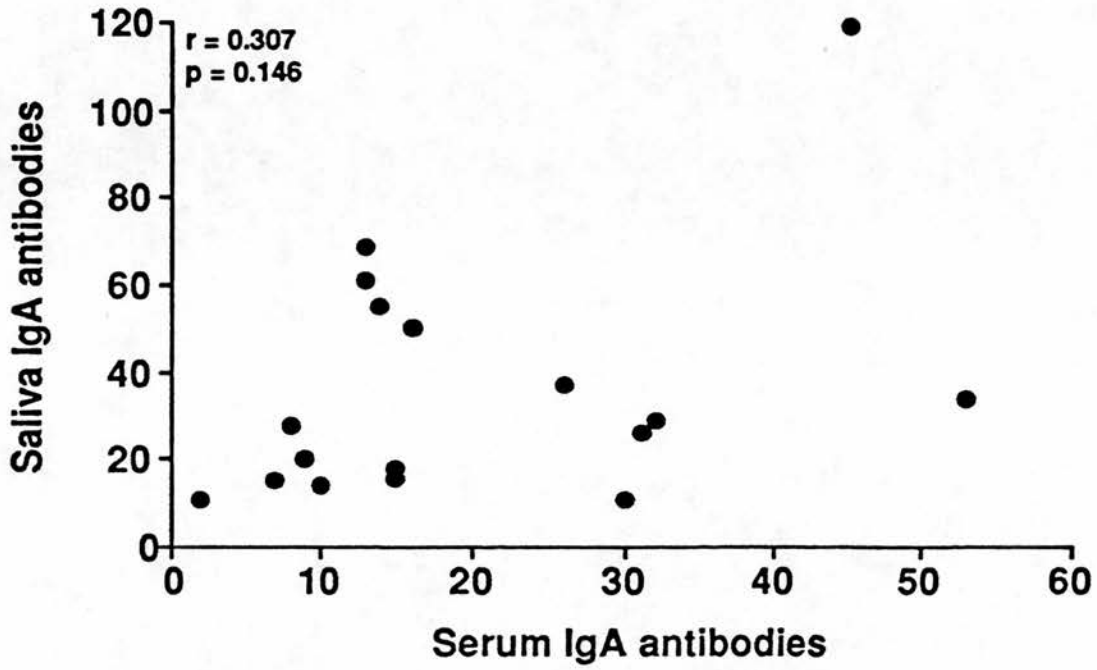
Graph 10A:3B

PAROTID SALIVA TOTAL IgG PLOTTED AGAINST SERUM TOTAL IgG



Graph 10A:3C

PAROTID SALIVA IgA ANTIBODIES PLOTTED AGAINST CORRESPONDING ANTIBODIES IN SERUM OF PATIENTS WITH POSITIVE SALIVA IgA ANTIBODIES



Graph 10A:3D

PAROTID SALIVA TOTAL IgA PLOTTED AGAINST SERUM TOTAL IgA

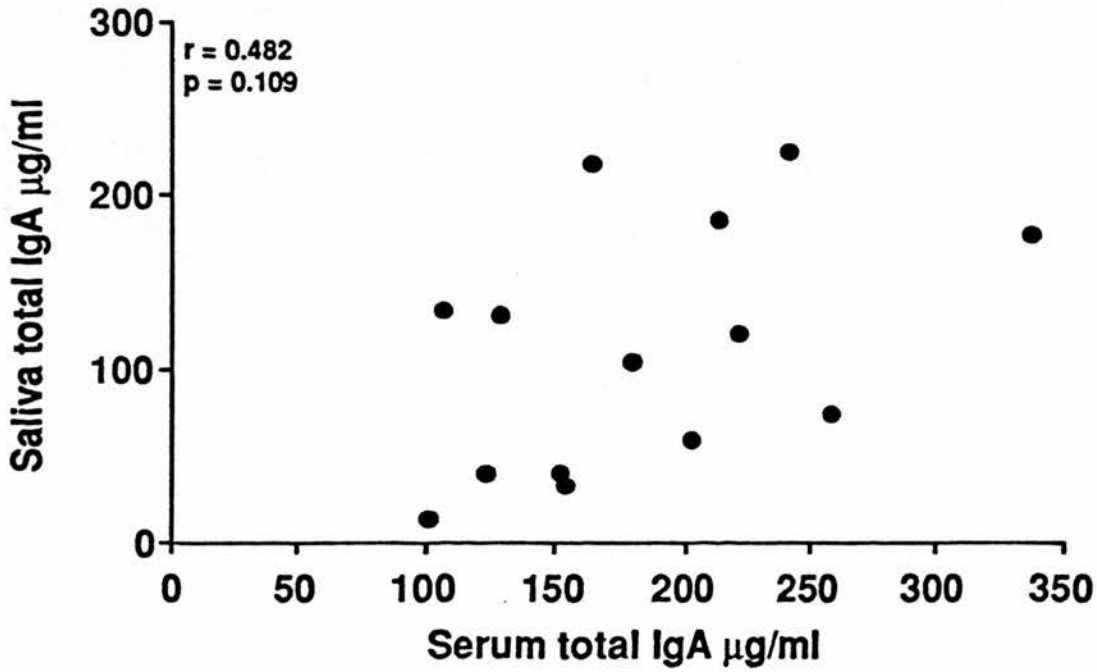


TABLE 10A:4A TOTAL SALIVA IMMUNOGLOBULINS (ug/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET.

	N	BEFORE MED (RANGE)	ON ED MED (RANGE)	AFTER ED MED (RANGE)
IgA	15	90 (15-205)	101 (14-186)	87 (12-240)
IgG	16	1.5 (1-19)	2 (1-34)	1.5 (1-49)
IgM	16	1.5 (1-8)	2 (1-13)	1.5 (1-7)

TABLE 10A:4B TOTAL SALIVA IMMUNOGLOBULINS (ug/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET IN RESPONDERS.

	N	BEFORE MED	ON ED MED	AFTER ED MED
IgA	6	113 (57-182)	123 (65-186)	76 (45-165)
IgG	6	1.5 (1-6)	1 (1-5)	1.0 (1-2)
IgM	6	1.5 (1-8)	1.5 (1-7)	1.0 (1-7)

TABLE 10A:4C TOTAL SALIVA IMMUNOGLOBULINS (ug/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET IN NON-RESPONDERS.

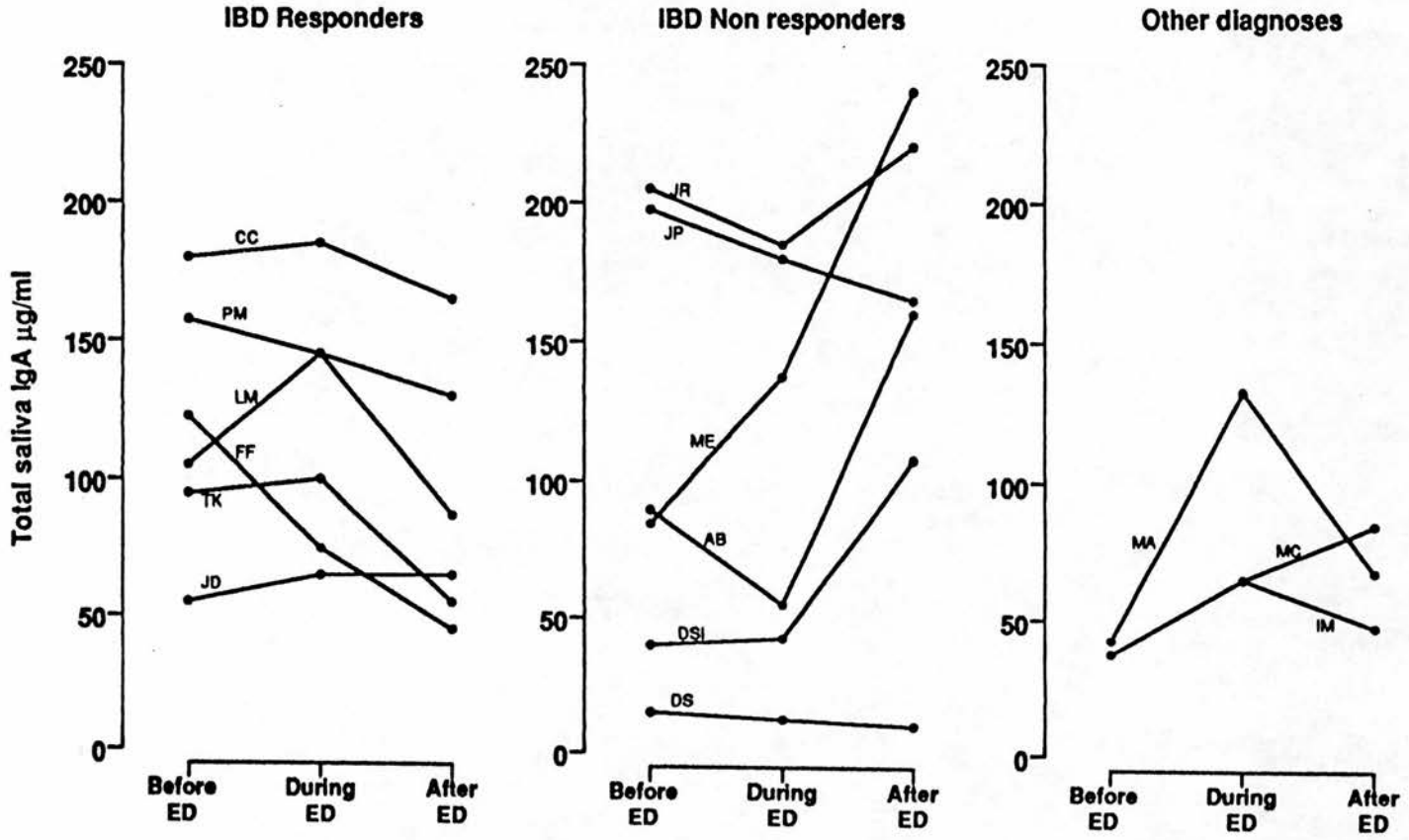
	N	BEFORE MED (RANGE)	ON ED MED (RANGE)	AFTER ED MED (RANGE)
IgA	9	42 (15-205)	65 (14-185)	108 (12-240)
IgG	10	4.5 (1-19)	2 (1-34)	3.5 (1-49)
IgM	10	1.5 (1-7)	2 (1-13)	2 (1-7)

TABLE 10A:4D TOTAL SALIVA IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IN ALL IBD PATIENTS. Statistical comparisons by Mann-Whitney (p values).

	IgA	IgG	IgM
BEFORE VS ON ED	0.443	0.295	0.666
BEFORE VS AFTER ED	0.670	0.471	0.824
ON ED VS AFTER ED	1.00	0.610	0.374

p= Probability that the difference is a chance finding

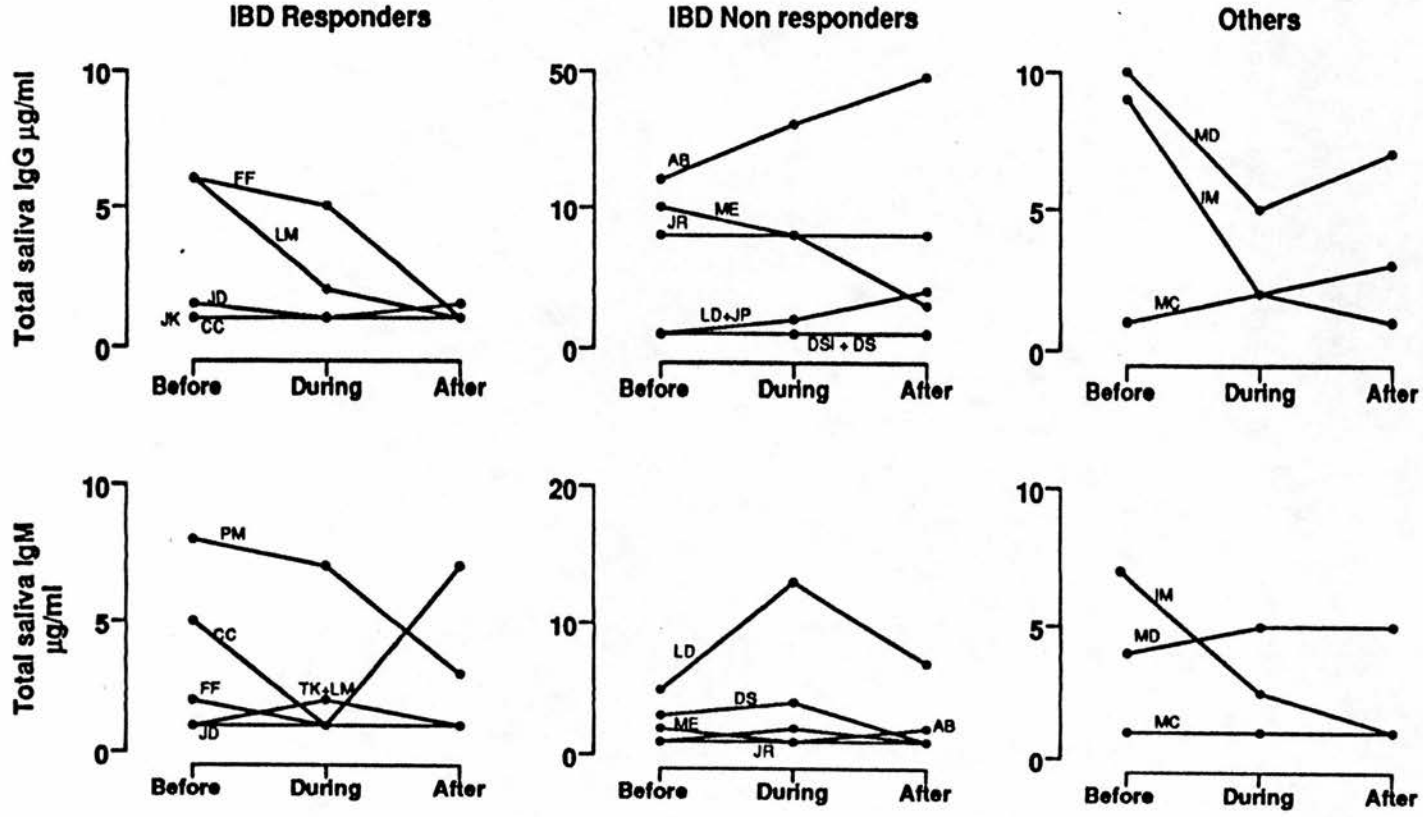
Graph 10A:4A
PAROTID SALIVA TOTAL IMMUNOGLOBULIN A AND ELEMENTAL DIET



Parotid saliva IgA immunoglobulin levels at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet (After). Responders and non-responders refers to IBD patients.
Others = non IBD IM - Coeliac MC - short bowel syndrome MD - irritable bowel syndrome

Graph 10A:4B

PAROTID SALIVA TOTAL IMMUNOGLOBULIN G AND M IN PATIENTS ON ELEMENTAL DIET



Parotid saliva IgG (Top) and IgM (bottom) levels at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet.
Others = non IBD patients. IM - Coeliac MD - irritable bowel syndrome MC - short bowel syndrome.

TABLE 10A:4E TOTAL SALIVA IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IN THE PATIENTS WHO RESPONDED. Statistical comparisons by Mann-Whitney (p values).

	IgA	IgG	IgM
BEFORE VS ON ED	1.000	0.181	0.590
BEFORE VS AFTER ED	0.059	0.371	0.789
ON ED VS AFTER ED	0.059	0.593	0.855

p= Probability that the difference is a chance finding

TABLE 10A:4F TOTAL SALIVA IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IBD THE PATIENTS WHO DID NOT RESPOND. Statistical comparisons by Mann-Whitney (p values).

	IgA	IgG	IgM
BEFORE VS ON ED	0.407	0.933	0.310
BEFORE VS AFTER ED	0.058	1.000	0.529
ON ED VS AFTER ED	0.236	0.310	0.355

p= Probability that the difference is a chance finding

TABLE 10A:5A SALIVA IgA FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET.

	BEFORE ED		ON ED	AFTER ED
	N	MED (RANGE)	MED (RANGE)	MED (RANGE)
ALL	19	36 (14-124)	30 (9-150)	20 (4-150)*
RES	10	55 (20-124)	29 (9-150)	25 (2-150)
NON RES	9	33 (14-79)	35 (11-69)	18 (11-60)*

RES - patients who improved clinically

NON-RES Patients who did not improve clinically

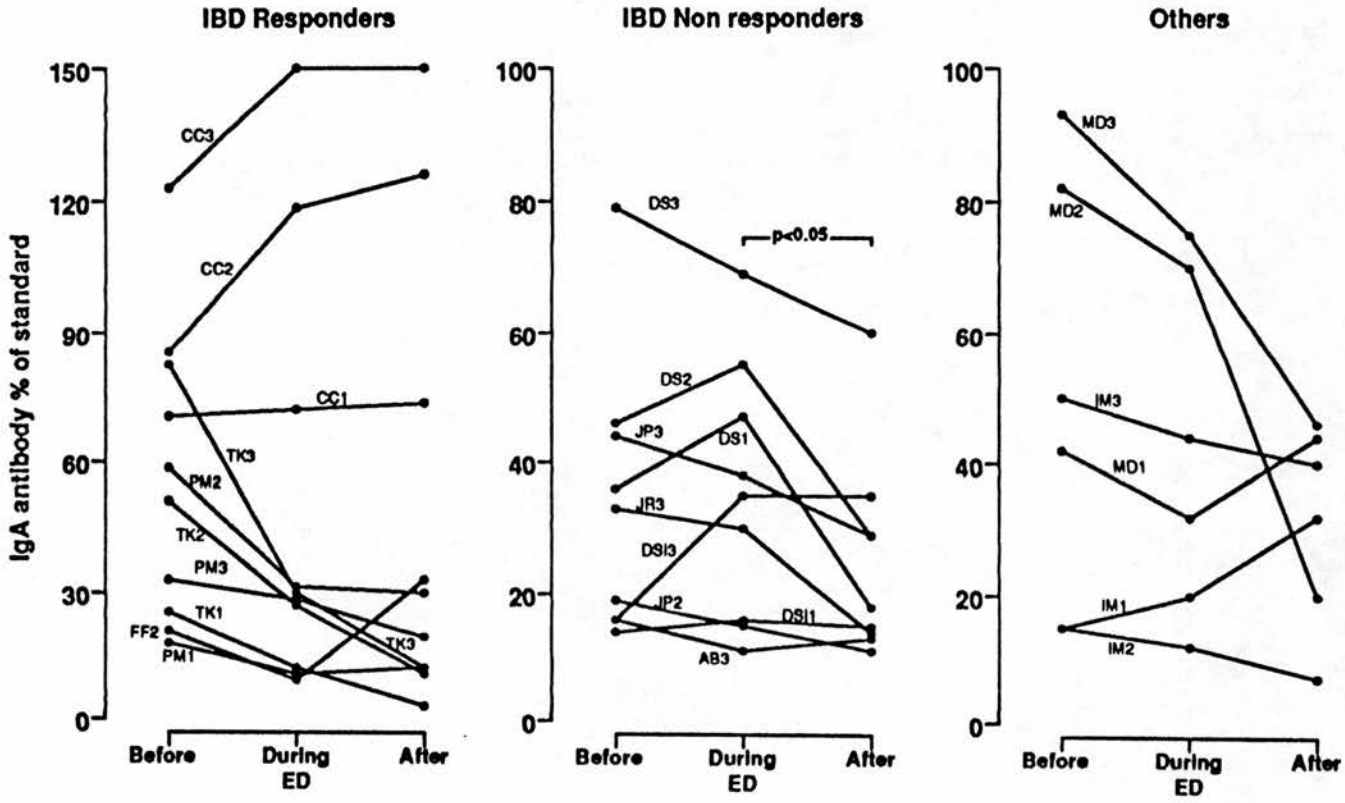
*Significantly lower than when on elemental diet

TABLE 10A:5B SALIVA IgA FOOD ANTIBODIES BEFORE, DURING AND AFTER ELEMENTAL DIET. Statistical analysis by Wilcoxon paired signed rank test (p values)

	ALL	RES	NON-RES
BEFORE VS ON ED	0.334	0.308	0.906
BEFORE VS AFTER ED	0.095	0.359	0.124
ON ED VS AFTER ED	0.031*	0.477	0.030*

p= Probability that the difference is a chance finding

Graph 10A:5
IgA FOOD ANTIBODIES IN PAROTID SALIVA OF PATIENTS ON ELEMENTAL DIET



Parotid saliva IgA antibodies at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet (After). Responders and non-responders refers to IBD patients.
1. Antigliadin 2. Anti-β-lactoglobulin 3. Antiovalbumin. Others = non IBD IM - Coeliac MD - irritable bowel syndrome

TABLE 10B:1A TOTAL SERUM IMMUNOGLOBULINS (IU/ml) OF ALL PATIENTS BEFORE, DURING AND AFTER ELEMENTAL DIET

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	23	132 (78-294)	116 (55-259)	130 (23-334)
ON ED	19	158 (16-333)	121 (64-334)	171 (31-373)
AFT ED	22	132 (64-272)	100 (53-261)	135 (31-361)

TABLE 10B:1B COMPARISONS OF TOTAL SERUM IMMUNOGLOBULINS OF ALL PATIENTS BEFORE, DURING AND AFTER ELEMENTAL DIET

Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.142	1.000	0.557
IgG	0.073	0.114	0.024*
IgM	0.147	0.426	0.218

p= Probability that the difference is a chance finding

TABLE 10B:1C TOTAL SERUM IMMUNOGLOBULINS (IU/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	20	133 (78-294)	112 (55-251)	155 (23-334)
ON ED	17	158 (16-333)	118 (64-205)	170 (31-373)
AFT ED	19	130 (64-272)	96 (53-261)	134 (31-361)

TABLE 10B:1D COMPARISON OF TOTAL SERUM IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS

Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.256	0.968	0.642
IgG	0.127	0.167	0.042*
IgM	0.124	0.277	0.349

p= Probability that the difference is a chance finding

TABLE 10B:1E TOTAL SERUM IMMUNOGLOBULINS (IU/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO RESPONDED

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	8	118 (78-181)	123 (55-160)	191 (23-311)
ON ED	7	120 (16-212)	134 (92-155)	188 (31-308)
AFT ED	7	115 (64-161)	100 (58-154)	153 (31-131)

TABLE 10B:1F COMPARISONS OF TOTAL SERUM IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO RESPONDED Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.554	0.933	0.834
IgG	0.310	0.447	0.295
IgM	0.353	0.800	0.418

p= Probability that the difference is a chance finding

TABLE 10B:1G TOTAL SERUM IMMUNOGLOBULINS (IU/ml) BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO DID NOT RESPOND

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	12	159 (108-294)	184 (101-333)	167 (104-272)
ON ED	10	97 (55-251)	104 (64-205)	92 (53-261)
AFT ED	12	90 (32-334)	126 (33-373)	115 (33-361)

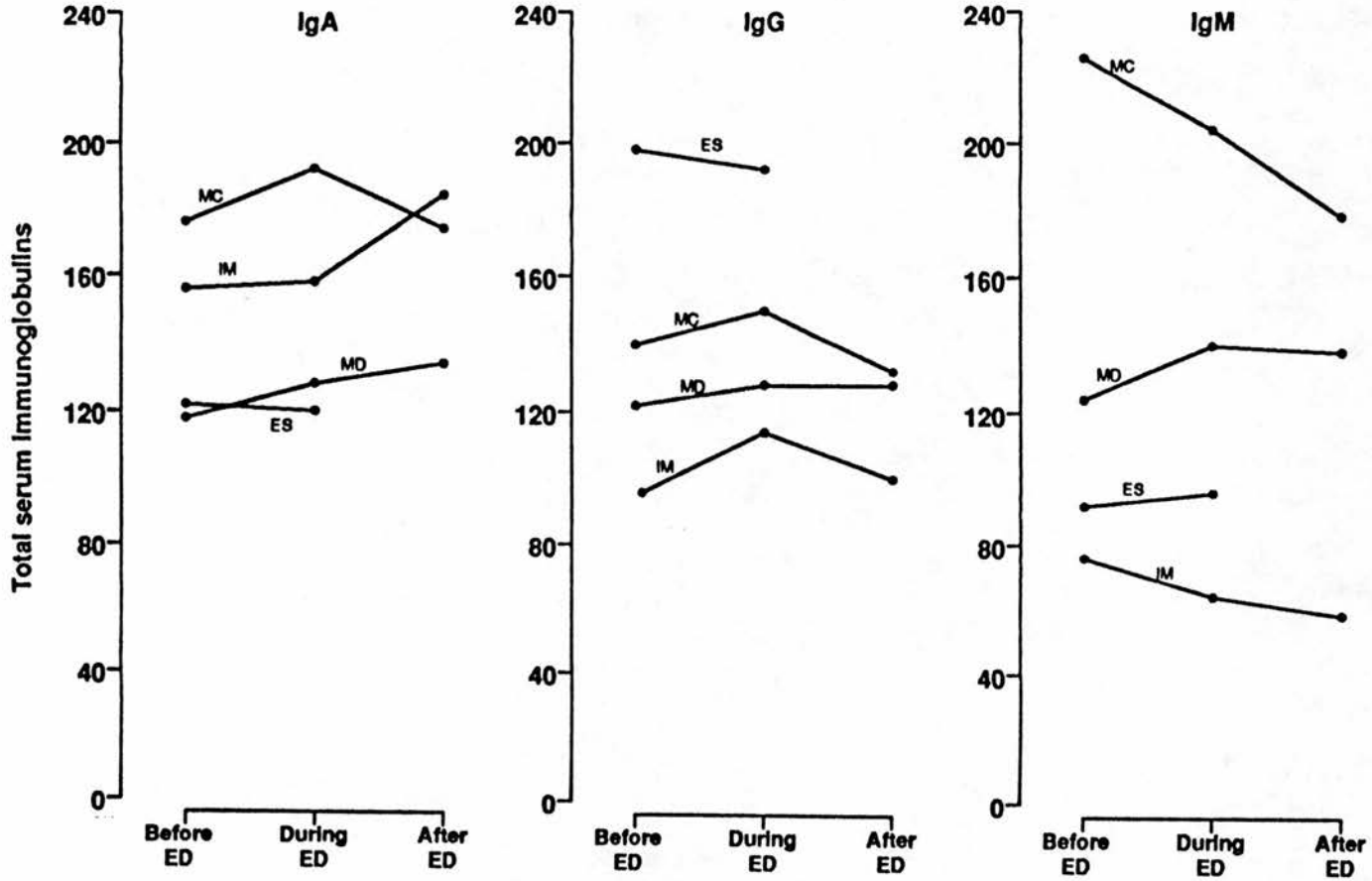
TABLE 10B:1H COMPARISONS TOTAL SERUM IMMUNOGLOBULINS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO DID NOT RESPOND Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.415	1.000	0.838
IgG	0.328	0.345	0.075
IgM	0.221	0.108	0.919

p= Probability that the difference is a chance finding

Graph 10B:1J

TOTAL SERUM IMMUNOGLOBULINS AND ELEMENTAL DIET IN NON-IBD PATIENTS



Total IgA, IgG and IgM in serum at three time points; before elemntal diet (Before), during (During) and after 4-7 days of normal diet. MC - short bowel syndrome IM and ES - Coeliac MD - Recurrent diarrhoea

TABLE 10B:2A SERUM FOOD ANTIBODY LEVELS OF ALL PATIENTS BEFORE, DURING AND AFTER ELEMENTAL DIET.

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
INIT	27	31 (21-93)	64 (41-165)	78 (45-142)
ON ED	20	38.5 (17-79)	62 (26-107)	79 (52-152)
AFT ED	27	31 (6-71)	64 (29-127)	66 (19-144)

TABLE 10B:2B COMPARISON OF SERUM FOOD ANTIBODY LEVELS OF ALL PATIENTS BEFORE, DURING AND AFTER ELEMENTAL DIET.

Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	INIvsAFT	EDvsAFT
IgA	0.514	0.119	0.010*
IgG	0.339	0.197	0.408
IgM	0.314	0.184	0.756

p= Probability that the difference is a chance finding

TABLE 10B:2C SERUM FOOD ANTIBODY LEVELS OF IBD PATIENTS BEFORE, DURING AND AFTER ELEMENTAL DIET

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	22	33.5 (21-93)	72 (41-165)	73 (45-142)
ON ED	15	43 (17-79)	64 (26-107)	70 (52-152)
AFT ED	22	34 (6-71)	64 (29-127)	66 (19-144)

TABLE 10B:2D COMPARISON OF SERUM FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS

Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.650	0.131	0.016*
IgG	0.311	0.082	0.142
IgM	0.800	0.589	0.554

p= Probability that the difference is a chance finding

TABLE 10B:2E SERUM FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO RESPONDED.

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	10	34 (26-47)	72 (49-80)	88.5 (62-142)
ON ED	8	38.5 (17-56)	65 (26-80)	86.5 (52-152)
AFT ED	10	28 (6-61)	60 (32-92)	96 (51-144)

TABLE 10B:2F COMPARISON OF FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO RESPONDED Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.402	0.476	0.234
IgG	0.855	0.554	0.584
IgM	1.000	1.000	0.855

p= Probability that the difference is a chance finding

TABLE 10B:2G SERUM FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO DID NOT RESPOND.

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	12	34 (21-93)	70 (41-165)	73 (45-101)
ON ED	7	45 (23-79)	64 (33-107)	70 (57-93)
AFT ED	12	38 (21-71)	65.5 (39-127)	66 (19-94)

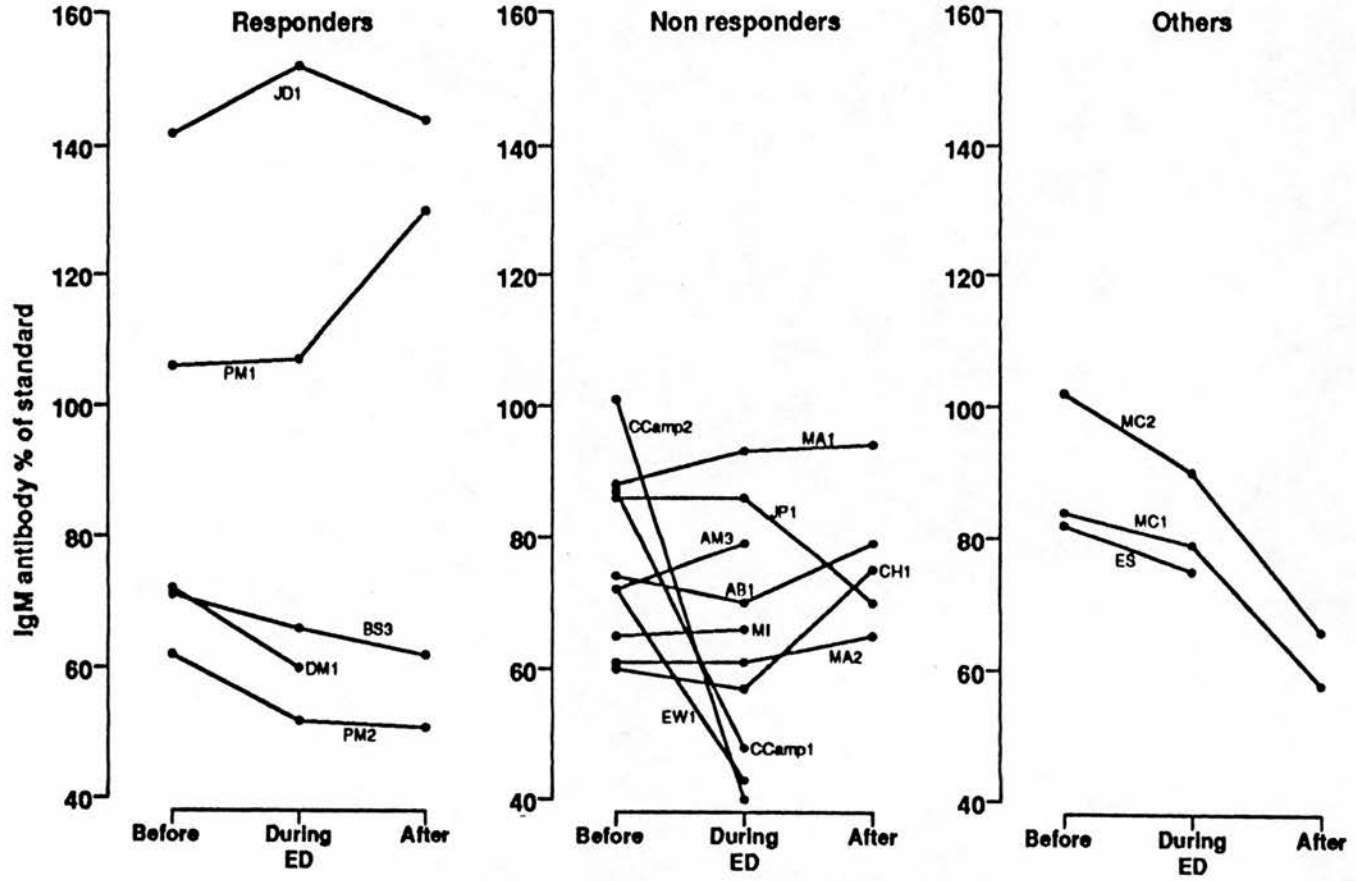
TABLE 10B:2H COMPARISON OF SERUM FOOD ANTIBODY LEVELS BEFORE, DURING AND AFTER ELEMENTAL DIET IN IBD PATIENTS WHO DID NOT RESPOND. Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFvsED	BEFvsAFT	EDvsAFT
IgA	0.735	0.241	0.022*
IgG	0.236	0.125	0.193
IgM	1.000	0.689	0.148

p= Probability that the difference is a chance finding

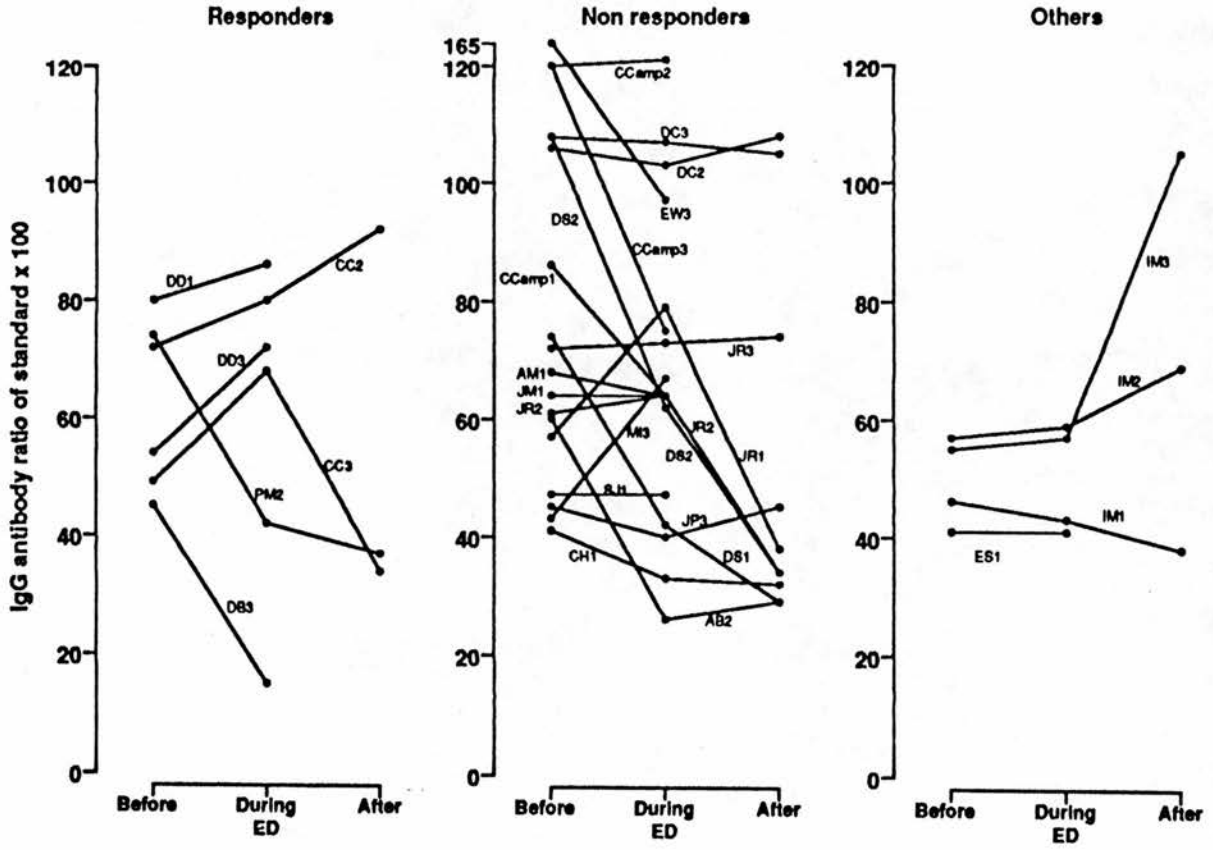
* Significantly lower than during elemental diet

Graph 10B:2F
ELEMENTAL DIET AND SERUM IgM ANTIBODIES



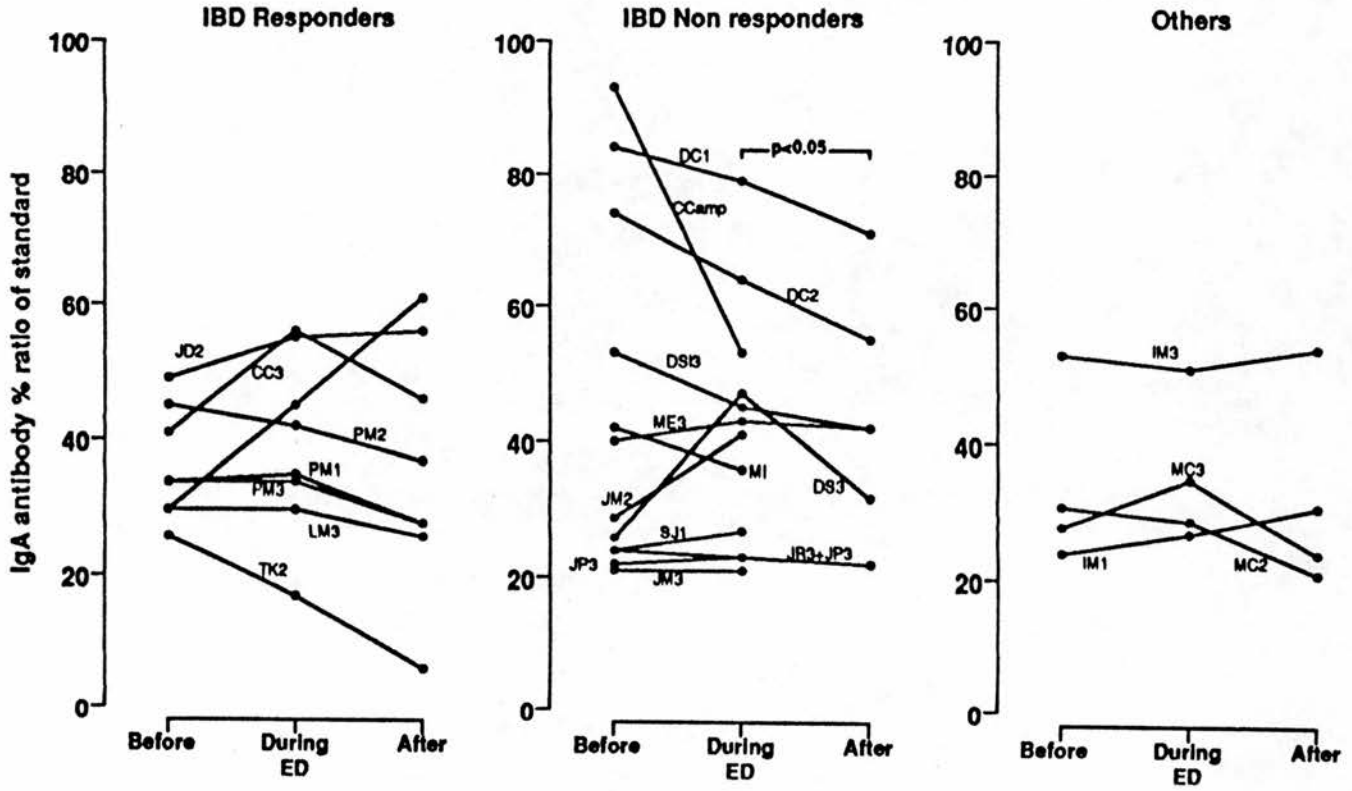
Serum IgM antibodies as a percentage of a known standard (Rankin = 100%) at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet (After). Responders and non-responders refers to IBD patients. 1. Antigliadin 2. Anti-β-lactoglobulin 3. Antiovalbumin. Others = non IBD MC - Short gut syndrome ES - Coeliac

Graph 10B:2G
ELEMENTAL DIET AND SERUM IgG ANTIBODIES



Serum IgG antibodies as a percentage of a known standard (Rankin = 100%) at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet (After). Responders and non-responders refers to IBD patients.
1. Antigliadin 2. Anti-β-lactoglobulin 3. Antiovalbumin. Others = non IBD patients, both coeliacs.

Graph 10B:2H
ELEMENTAL DIET AND SERUM IgA ANTIBODIES



Serum IgA antibodies as a percentage of a known standard (Rankin = 100%) at three time points; before elemental diet (Before), during elemental diet (During) and 4 to 7 days after returning to normal diet (After). Responders and non-responders refers to IBD patients. 1. Antigliadin 2. Anti-β-lactoglobulin 3. Antiovalbumin. Others = non IBD IM - Coeliac MC - Short gut syndrome post surgery for tuberculosis

TABLE 10C:1A

FOOD ANTIBODY SECRETING CELLS AND ELEMENTAL DIET

						CLINICAL DATA															
NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	TP	ALB	HB	CRP	ESR	WT	TP	ALB				
LM	23	F	1(LC)	ACUTE EXACERBATION	NONE	15	9.2	6.9	68	52	65	34	10	1.5	45	53	63	34				
SS	23	F	1(LC)	ACUTE EXACERBATION	ILEUM + COLON	8	12	1.5	11	41	68	42	11	1.5	14	42	74	45				
DC	24	M	1(LC)	ACUTE EXACERBATION	ILEUM	21	16.4	3.1	14	–	71	35	17	1.5	3	–	68	34				
CH	70	F	1	ACUTE EXACERBATION	ILEUM + CAECUM	9	12	10	96	56	72	35	11	15	102	56	67	31				
DM	29	F	1	STEROID FAILURE	NONE	21	13	2.3	37	50	71	36	13	1.5	15	49	71	38				
DD	28	F	1	ACUTE EXACERBATION	TRANSVERSE COLON + ILEUM	9	11	6.6	21	–	54	28	11	1.5	13	–	54	27				
MA	34	F	1	DIARRHEA	NONE	10	15	–	1	62	67	40	15	–	1	62	71	42				
BS	28	M	2	STEROID FAILURE	NONE	10	11	10	90	46	64	34	11	1.5	50	51	64	34				
ES	34	F	3	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	80	43	12	–	7	40	75	43				

*← BEFORE ELEMENTAL DIET → **← ON ELEMENTAL DIET → *

ABBREVIATIONS

1 = ILEAL CROHN'S DISEASE

ILC = ILEOCOLONIC CROHN'S DISEASE

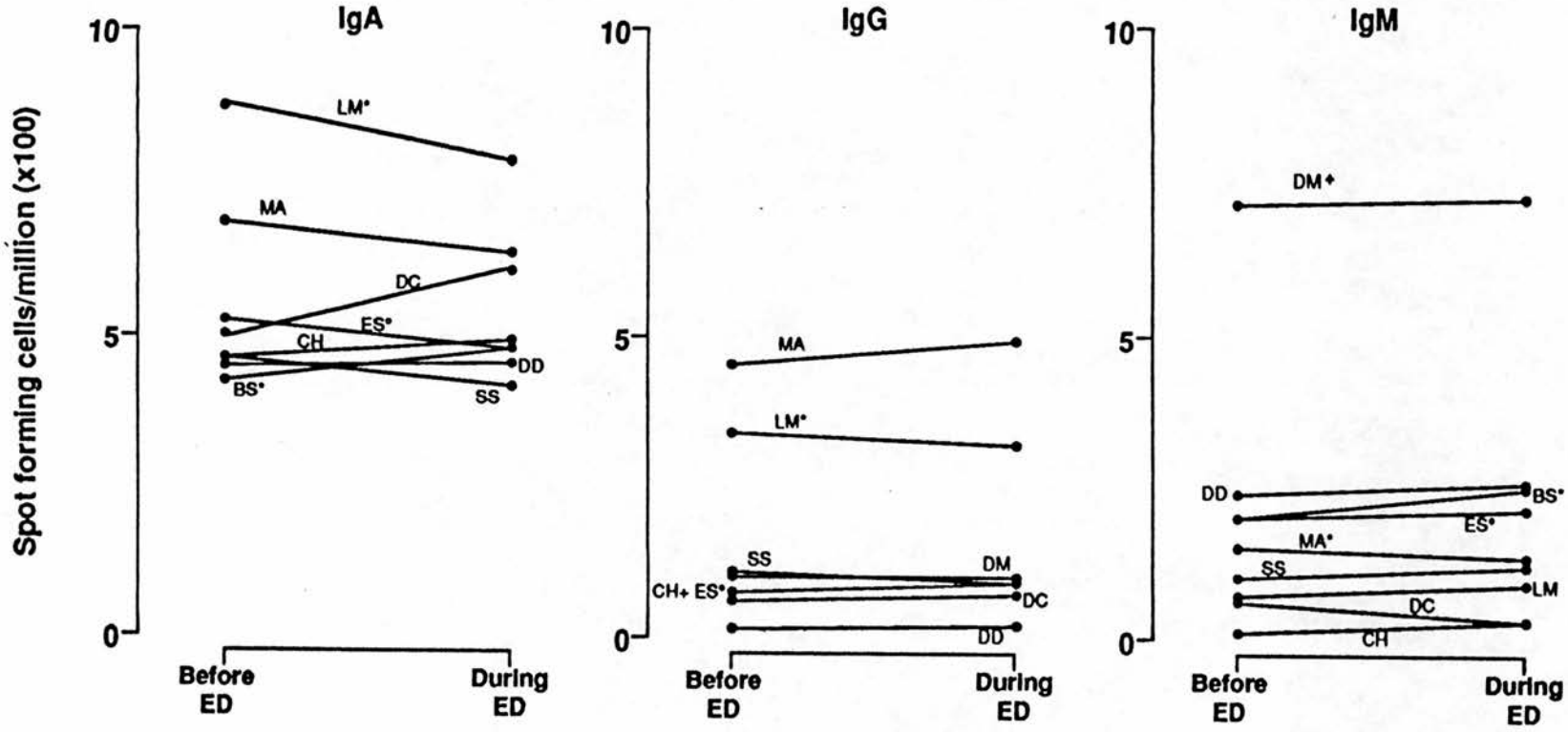
2 = ULCERATIVE COLITIS

3 = COELIAC DISEASE

DOD = DAYS ON DIET

Graph 10C:1A

IMMUNOGLOBULIN SECRETING CELLS AND ELEMENTAL DIET



IgA, IgG and IgM secreting cells in peripheral blood (ELISPOT) before and after at least seven days on elemental diet. * = patients who improved. All patients had Crohn's disease except BS - ulcerative colitis and ES - Coeliac disease. DM+ - IgA deficient

TABLE 10C:1B THE NUMBERS OF IMMUNOGLOBULIN SECRETING CELLS PER MILLION LYMPHOCYTES IN PERIPHERAL BLOOD BEFORE AND DURING ELEMENTAL DIET.

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE ED	9	465 (440-890)	100 (14-460)	101 (10-733)
DURING ED	9	495 (98-800)	97 (20-506)	128 (30-740)

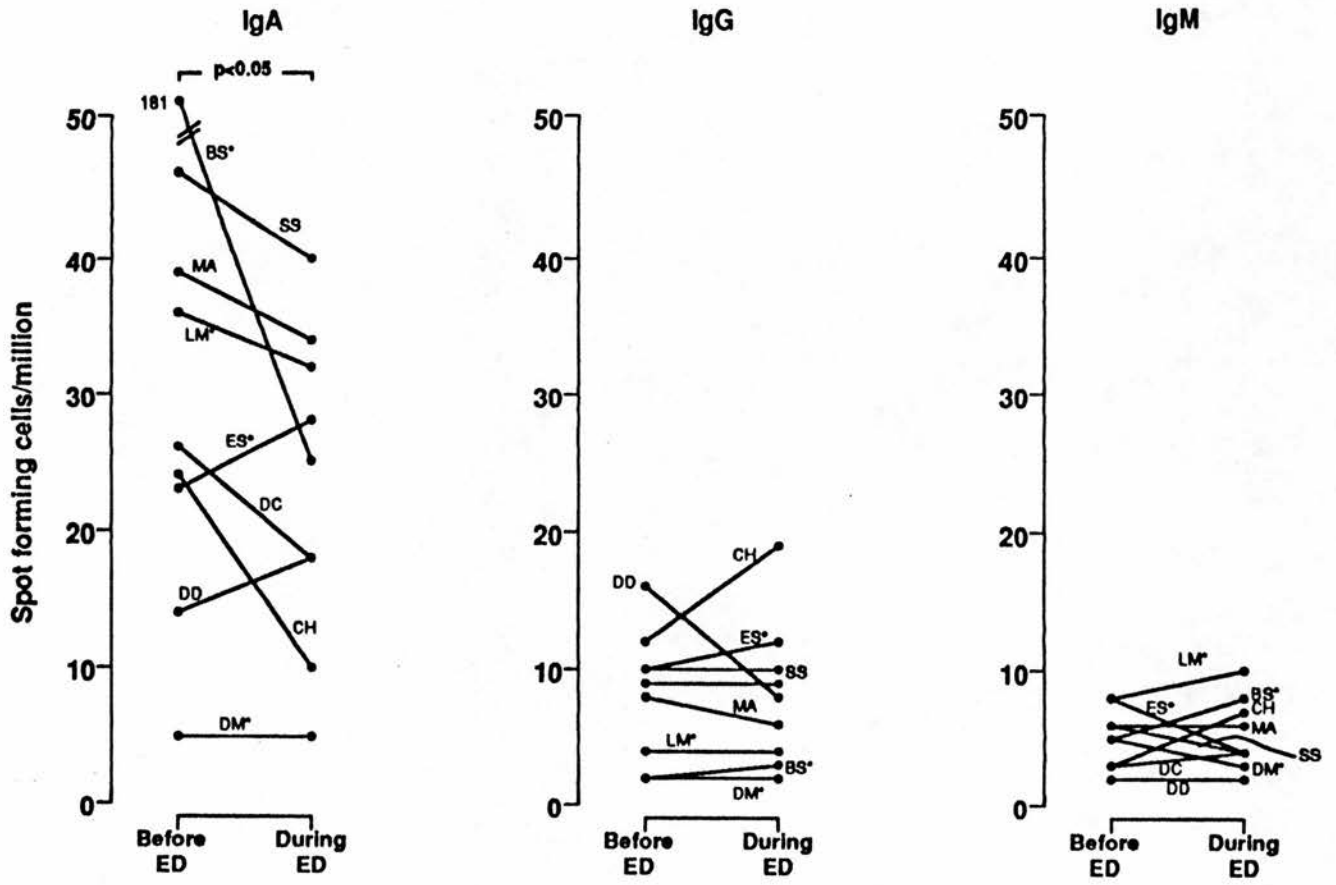
TABLE 10C:1C COMPARISON OF THE NUMBERS OF IMMUNOGLOBULIN SECRETING CELLS IN PERIPHERAL BLOOD BEFORE AND DURING ELEMENTAL DIET. Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFORE vs DURING
IgA	1.000
IgG	0.675
IgM	0.441

p= Probability that the difference is a chance finding

Graph 10C:2A

ANTIBODY SECRETING CELLS IN PERIPHERAL BLOOD AND ELEMENTAL DIET



Antibody secreting cells in peripheral blood (ELISPOT ASSAY) of patients before and after at least 7 days on elemental diet.
All patients had Crohn's disease except ES who was coeliac and BS who had ulcerative colitis.
* = patients who improved DM is IgA deficient

TABLE 10C:2A COMPARISON OF NUMBERS OF ANTIBODY SECRETING CELLS DIET IN PERIPHERAL BLOOD OF IBD PATIENTS BEFORE AND DURING ELEMENTAL DIET Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFORE vs DURING
IgA	0.043*
IgG	0.789
IgM	1.000

p= Probability that the difference is a chance finding

TABLE 100:1A

WGLF-FOOD ANTIBODIES AND ELEMENTAL DIET

CLINICAL DATA

NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	TP	ALB	LTIGG	HB	CRP	ESR	WT	TP	ALB	TIGG	CO
DM	29	F	1	STEROID FAILURE	NONE	21	14	2.3	37	50	71	36	64	13	1.5	15	49	71	38	46	*
EM	51	F	1	SB OBSTRUCTION RELUCTANT TO TAKE STEROIDS	TERMINAL ILEUM	14	13	11	79	57	73	40	29	12	16	92	60	73	40	22	N
DB	19	M	1	RECURRENT ACTIVE CROHN'S	ILECOLECTOMY	21	11	5.5	63	52	79	36	3	10	1.5	37	52	76	34	1	*
AM	38	F	1(LC)	OBSTRUCTIVE SYMPTOMS CROHN'S DISEASE	PROCTOCOLECTOMY	21	13	3.6	34	68	69	36	19	15	1.5	17	68	76	42	12	N
DS	30	F	1	RECURRENT ACTIVE DISEASE	HEMICOLECTOMY	31	10	3.6	25	42	51	30	64	11	3.7	32	44	59	31	61	N
MI	50	F	1(LC)	ACTIVE ILEAL DISEASE	RT HEMICOLECTOMY	21	14	1.5	15	–	64	31	1	13	1.5	11	–	57	30	1	D
JM	54	F	1(COL)	RECURRENT ACTIVE DISEASE	SIGMOID RESECTION	14	11	10	46	66	65	32	15	10	1.5	42	64	66	33	12	D
CC	16	M	1(PA)	PERSISTENTLY ACTIVE CROHN'S DISEASE	SUBTOTAL COLECTOMY	14	10	7	25	25	74	28	47	15	1.5	33	25.5	78	31	19	D
SJ	26	M	1(COL)	ABDO. PAIN AND DIARRHOEA	NONE	21	14	1.5	33	82	82	40	2	14	1.5	13	83	83	40	5	D
EW	25	F	1(COL)	RECURRENT ACTIVE DISEASE	NONE	14	11	2.0	83	45	64	34	133	9.5	3.9	80	45	64	34	131	D
BS	28	M	2	STEROID FAILURE	NONE	10	11	10	90	46	64	34	120	11	1.5	50	51	64	34	120	*
FK	44	F	1	ACTIVE ILEAL DISEASE	RT HEMICOLECTOMY	14	10	1.5	25	41	66	38	11	12	1.5	10	41	72	44	2	N
ES	34	F	3	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	80	43	3	12	1.5	10	41	75	44	2	N

←BEFORE ELEMENTAL DIET——→ * ←—— ON ELEMENTAL DIET→*

ABBREVIATIONS

1 = ILEAL CROHN'S DISEASE

2 = ULCERATIVE COLITIS

3 = COELIAC DISEASE

1(LC) = ILEOCOLONIC CROHN'S DISEASE

1(COL) = COLONIC CROHN'S DISEASE

DOD = DAYS ON DIET

LTIGG = LAVAGE TOTAL IgG

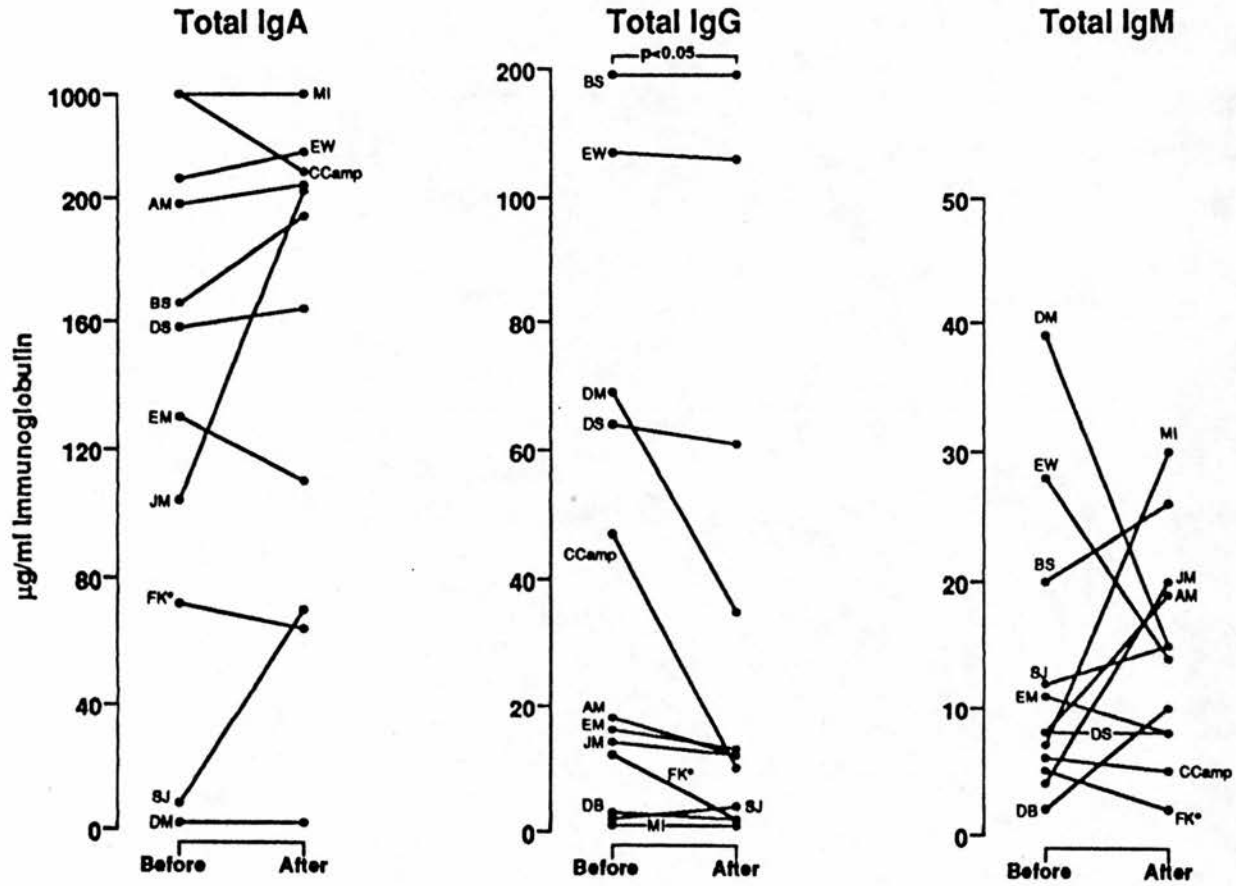
CLINICAL OUTCOME (CO)

* = RESPONDERS

D = DETERIORATED

N = NO CHANGE

Graph 10D:1A
WHOLE GUT LAVAGE IMMUNOGLOBULINS AND ELEMENTAL DIET



Total immunoglobulins in whole gut lavage fluid before and after at least 7 days on elemental diet. FK° - Triosorbin. No significant fall in total immunoglobulins except for total IgG.

TABLE 10D:1B WGLF TOTAL IMMUNOGLOBULINS (ug/ml) BEFORE AND DURING ELEMENTAL DIET IN IBD PATIENTS ONLY

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	10	162 (3-988)	19 (1-196)	8 (2-39)
DURING	11	203 (3-990)	12 (1-196)	15 (5-30)

TABLE 10D:1C COMPARISON OF WGLF TOTAL IMMUNOGLOBULINS BEFORE AND DURING ELEMENTAL DIET IN IBD PATIENTS ONLY

Statistical analysis by Wilcoxon paired signed rank test (p values)

	DURING vs AFTER
IgA	0.813
IgG	0.044*
IgM	0.154

p= Probability that the difference is a chance finding

TABLE 10D:2A WGLF FOOD ANTIBODIES BEFORE AND DURING ELEMENTAL DIET.

	N	IgA MED (RANGE)	IgG MED (RANGE)	IgM MED (RANGE)
BEFORE	16	31 (12-87)	36 (31-42)	21 (11-51)
DURING	15	36 (11-98)	3 (2-22)	17 (6-36)

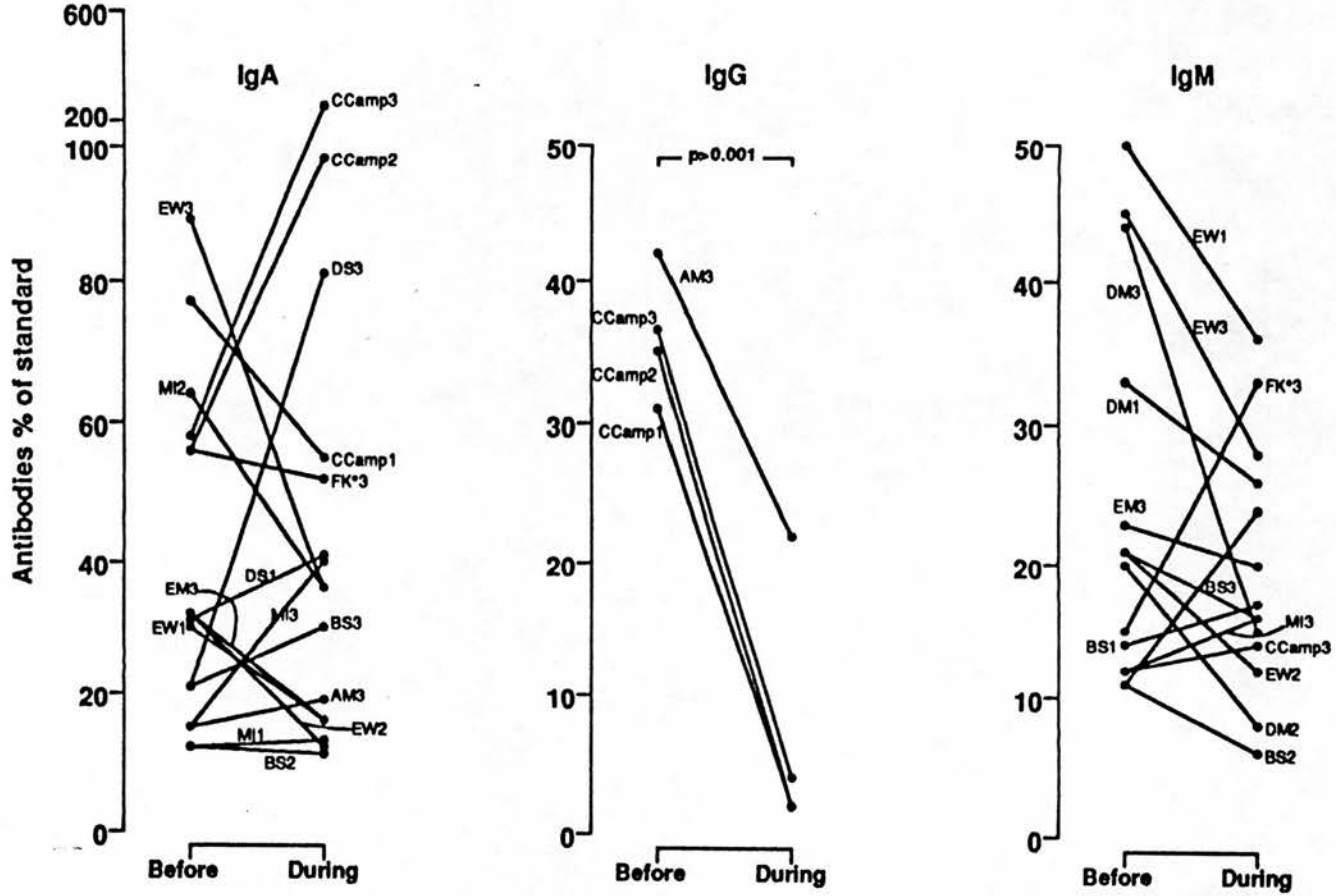
TABLE 10D:2B COMPARISON OF WGLF FOOD ANTIBODIES BEFORE AND DURING ELEMENTAL DIET. Statistical analysis by Wilcoxon paired signed rank test (p values)

	BEFORE vs DURING
IgA	0.875
IgG	0.001*
IgM	0.087

p= Probability that the difference is a chance finding

Graph 10D:2A

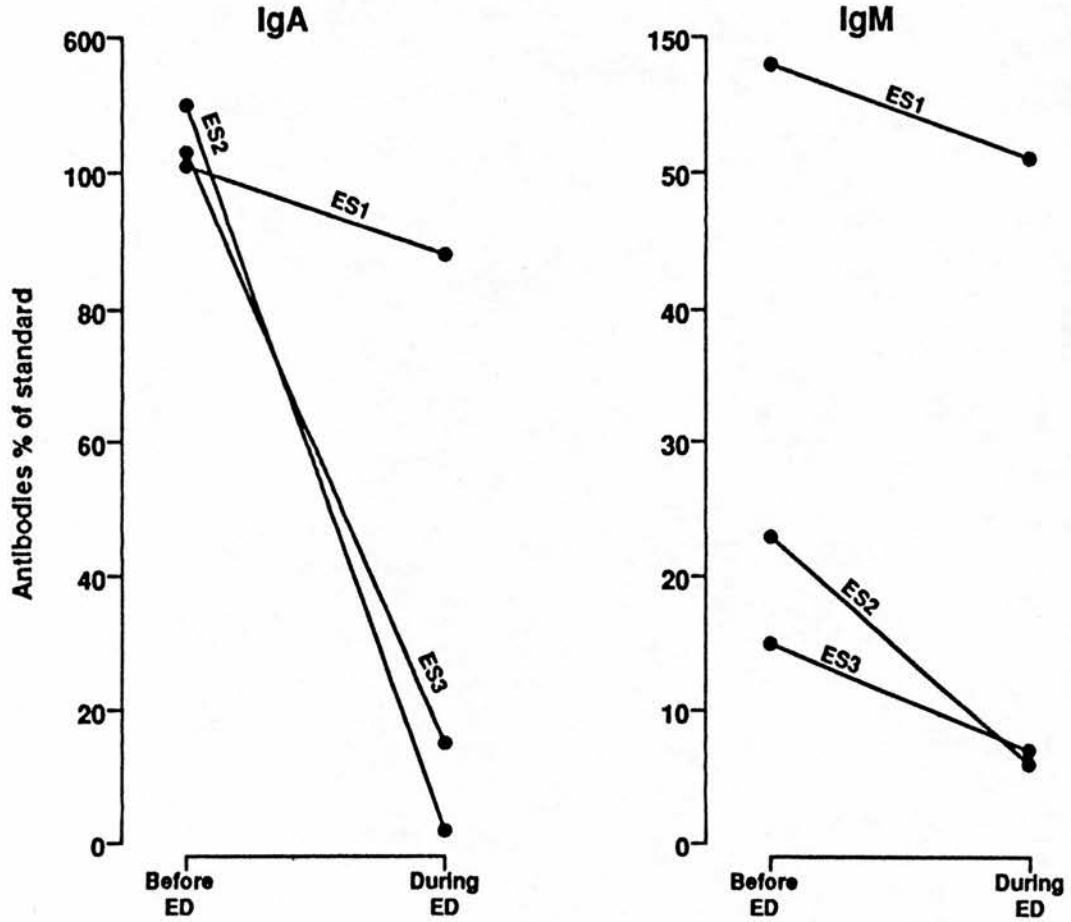
WHOLE GUT LAVAGE FLUID FOOD ANTIBODIES AND ELEMENTAL DIET



Whole gut lavage food antibodies (IgA, IgG, IgM) as a percentage of a known standard (Rankin = 100%) before and after at leaset 7 days on elemental diet. 1. Antigliadin 2. Anti-β-lactoglobulin 3. Antiovalbumin. FK° - Triosorbin

Graph 10D:2B

WHOLE GUT LAVAGE FLUID FOOD ANTIBODIES AND ELEMENTAL DIET NON-IBD PATIENT (COELIAC)



Whole gut lavage antibodies (IgA and IgM) as a percentage of a known standard (Rankin = 100%) at two time points; before elemental diet (Before ED) and after at least 7 days of elemental diet.

CHAPTER ELEVEN:

ALPHA-1 ACID GLYCOPROTEIN

INTRODUCTION

The level of alpha-1 acid glycoprotein (α-1AP) has been used as an index of disease activity in patients with inflammatory bowel disease (see chapter 3). It has also been found to be a good predictor of relapse of inflammatory bowel disease (Wright et al., 1987).
A combination of IL-1, IL-6 and TNF acting sequentially increase acid glycoprotein production though individually each induces a different set of acute phase proteins (Emery and Salmon, 1991). As these systemically acting cytokines can be produced in combination and in abundance by macrophages, macrophage cytokine activity may be studied via alpha-1 acid glycoprotein.

THE AIM OF THIS STUDY

The aims of this study were firstly to establish the pattern of α-1 acid glycoprotein in serum and whole gut lavage fluid (WGLF) of controls and patients with inflammatory bowel disease and secondly, to establish if α-1AP levels were associated with response or lack of response to elemental diet therapy.

PATIENTS AND PROTOCOL

A total of 51 patients with inflammatory bowel disease (28 Crohn's disease and 23 ulcerative colitis) and 21 controls were studied (Table 11:1A).

Of the 28 patients with Crohn's disease the macroscopic disease in this group at the time of lavage was as follows: small bowel (13) (4 jejunal and 9 terminal ileum) ileocolonic (5), colonic (6), rectal (2) (one associated with anal disease), perianal (1) and one with anal disease. Five had undergone resections. All the patients were on normal diet. As regards drug treatment 4 patients were on steroids the dose ranging between 5-40mg per day, 7 on sulphasalazine, 3 on sulphasalazine and steroids, 1 on prednisolone and colifoam enemas, 2 on 5-amino salicylates only , 1 on prednisolone and colifoam and 10 on no drug therapy for their Crohn's disease. The age range was 13-82 with a median of 39. Eighteen had active disease and in 10 the disease was inactive as assessed clinically and also based on WGLF total IgG (see chapter 7).

None of the 23 patients with ulcerative colitis had had any resections. The macroscopic distribution of the disease at time of sampling was as follows: 10 pancolitis, 4 left-sided involvement, 7 proctitis, 1 UC pouchitis and 1 with microscopic ulcerative colitis. Seven were on no drug treatment, 3 were on oral

prednisolone and an amino-salicylate (ASA) type drug plus rectal steroids, 2 were on an ASA group drug plus rectal steroids, 1 was on rectal steroids, 4 on an oral ASA group only, 2 were taking oral prednisolone as well as ASA, 1 was on a combination of colifoam, azathioprine and ASA, 2 on prednisolone only and 1 was on cyclosporin in addition to oral steroids and ASA. The age range for this group was 22-78 with a median of 37. Of the 23, 13 had active disease at time of study.

The final diagnoses in the controls were as follows: non-inflammatory polyps in the colon (5), constipation (4), diarrhoea with no abnormality detected (1), duodenal ulcer (1), unexplained abdominal pain (5), colonic angiodysplasia (1), megacolon (1) and 3 healthy controls. The age range was 20-79 with a median of 47. All controls were on normal diet

Serum and WGLF α-1 acid glycoprotein were measured by immunoturbidimetry (described in chapter 6A).

Whole gut lavage fluid was collected from all the patients. Serum specimens were obtained from 16 controls, 21 of the 23 patients with ulcerative colitis, 26 of the 28 patients with Crohn's disease and the four gluten enteropathy patients. The collections were done on the same day so that the two specimens reflected systemic and mucosal α-1 acid glycoprotein activity at corresponding times.

Comparisons in the levels α-1 acid glycoprotein in serum

(SGLY) and WGLF (LGLY) were made between controls and the IBD patients. Comparisons were also made when the disease groups were further subdivided according to whether they had active or inactive disease and according to the regional distribution of disease.

Correlations analyses between the levels of α-1 acid glycoprotein in sera and gut lavage fluid were performed to define their relationship if any.

Finally levels of serum and lavage α-1 acid glycoprotein were measured in patients who were prescribed elemental diet, before and after seven or more days on elemental diet.

SECTION 11A

SERUM α-1 ACID GLYCOPROTEIN LEVELS IN CONTROLS AND PATIENTS WITH IBD

A total of 16 sera from controls were assayed for α-1AP. The controls gave a range of 0.19-0.73mg/ml with a median of 0.40 (Table 11A:1B). A level of 0.69mg/ml was two standard deviations above the median and considered high for further analysis of high values within groups.

The sera from the 21 patients with ulcerative colitis gave a range of 0.42-5.40mg/ml with a median of 1.60, and this was significantly higher than the control levels ($p = 0.00001$). The 26 serum specimens from Crohn's disease patients gave a range of 0.05-7.5mg/ml with a median

of 3.90mg/ml, and this was significantly higher than controls (p=0.00001) [Table 11A:1B; Table 11A:1C and Graph 11:1].

DISEASE ACTIVITY AND SERUM α-1 ACID GLYCOPROTEIN

Patients were subgrouped according to disease activity based on clinical assessment and WGLF total IgG. Patients with active ulcerative colitis gave a range of 0.42-5.40mg/ml with a median of 1.9mg/ml, which was significantly higher than controls (p=0.004). Active Crohn's patients gave a range of 0.05-7.50 with a median of 5.25mg/ml, and this was significantly higher than controls (p=0.00001) [Table 11A:1C; Table 11A:1D and Graph 11:1].

The range for inactive ulcerative colitis was 0.80-4.2mg/ml with a median of 1.32mg/ml, significant higher than controls (p=0.029). Inactive Crohn's gave a range of 0.85-7.3mg/ml with a median of 2.70mg/ml, significantly higher than controls (p=0.0001) [Table 11A:1C and Table 11A:1E].

A COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN BETWEEN DISEASE GROUPS

There was no significant difference in serum α-1AP between Crohn's disease and ulcerative colitis or between

active and inactive Crohn's. There was no significant difference either between active and inactive ulcerative colitis or inactive ulcerative colitis and inactive Crohn's disease. Serum α-1 acid glycoprotein was significantly higher in active Crohn's disease than in patients with active ulcerative colitis (p=0.0032; Table 11A:1F).

COMMENT

Serum levels of α-1 acid glycoprotein in the controls in this study were low compared to other reports even though there has been disagreement about the normal levels with ranges between the lowest reported 0.45-1.70mg/ml (de la Heuerga et al., 1956) and the highest 0.83-2.03 (Lockey et al., 1956) and some intermediate levels. Despite the lower levels in the controls in this study the patients with IBD had unequivocally high levels hence the significant differences with all the disease groups.

Though the differences between active and inactive disease groups did not reach statistical levels of significance, the active disease groups (means, 4.58mg/ml and 1.94mg/ml for active Crohn's disease and active ulcerative colitis respectively; p=0.00001) had higher means and levels of significance against controls

compared to inactive disease groups (means, 3.22mg/ml and 1.86mg/ml for inactive Crohn's disease and inactive ulcerative colitis respectively; p=0.0001). This though suggesting that serum α-1 acid glycoprotein increases with disease activity shows that it does not distinguish clearly between active and inactive disease.

Comparisons between disease groups shows that active Crohn's disease has higher levels than active ulcerative colitis (p=0.0032), this could be because systemic manifestations such as arthritis or uveitis are more prevalent in patients with Crohn's disease.

REGIONAL INVOLVEMENT OF DISEASE AND SERUM α-1 ACID GLYCOPROTEIN

Patients with Crohn's disease were further subdivided according to regional disease involvement into two groups. Patients with disease confined to the small bowel with no colonic involvement were grouped as small bowel (SB) Crohn's disease. Patients with disease confined to the colon and no small bowel involvement were grouped as colonic (CN) Crohn's disease. This classification excluded the five patients with ileocolonic involvement.

Comparisons of the serum levels of α-1 acid glycoprotein were made between the ulcerative colitis group, colonic Crohn's disease group and the small bowel Crohn's disease group to ascertain whether raised α-1AP was a feature of

small bowel or large bowel disease.

The small bowel Crohn's gave a range of 0.05-7.5mg/ml with a median of 5.25mg/ml. Colonic Crohn's gave a range of 1.28-7.40mg/ml with a median of 4.49mg/ml not significantly different from small bowel Crohn's p=1.0000. Patients with ulcerative colitis gave a range of 0.42-5.40mg/ml with a median of 1.60mg/ml and this was significantly lower than the levels for small bowel Crohn's disease p=0.0079 (Table 11A:2A). The prevalence of high levels of serum α-1AP was also analysed. As table 11:2B shows 10 out of 11 patients with small bowel Crohn's had high levels compared to 10 out 10 patients with colonic Crohn's disease. Though out of a total of 21 patients with ulcerative colitis 20 had high levels (>0.69mg/ml) these were statistically lower than those of patients with small bowel disease (Table 11A:2B).

REGIONAL INVOLVEMENT OF DISEASE AND SERUM α-1 ACID GLYCOPROTEIN LEVELS ACCORDING TO DISEASE ACTIVITY.

Patients were further subgrouped according to disease activity based on clinical assessment and WGLF total IgG. Active small bowel disease gave a range of 0.05-7.50mg/ml with a median of 5.25mg/ml. Active colonic Crohn's gave a range 1.28-7.40mg/ml with a median of 5.14mg/ml, not significantly different from active small bowel involvement p=0.8170. Active ulcerative colitis gave a

range of 0.42-5.40mg/ml with a median of 1.90mg/ml, and this was significantly less than for small bowel Crohn's disease p=0.0312 (Table 11A:2C).

There were only three patients with inactive small bowel Crohn's disease and 2 with inactive colonic Crohn's disease, numbers inadequate for firm statistical evaluations. This is why the figures for the whole subgroups are the same as for the patients with active disease only.

A COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN LEVELS BETWEEN COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS

Patients with colonic Crohn's disease had significantly higher levels of serum α-1AP than patients with ulcerative colitis (p=0.0031), as had patients with active colonic Crohn's disease compared to patients with active ulcerative colitis (p=0.0038). There were only two patients with inactive colonic Crohn's disease and these were not analysed further (Table 11A:2D).

COMMENT

These results show that there is no difference in α-1 acid glycoprotein activity between small bowel and large bowel Crohn's disease. This may be explained by the fact that most of the serum α-1 acid glycoprotein is of

hepatic origin as an acute phase reactant, so it is the overall systemic effect via the liver other than local regional variations that would influence the serum levels (Emery and Salmon, 1991). Both small bowel Crohn's and colonic Crohn's have significantly higher levels of α-1AP in serum than ulcerative colitis ($p=0.0079$ and $p=0.0031$ respectively). Patients with active Crohn's disease also showed higher levels of serum α-1AP than patients with acute ulcerative colitis ($p=0.0312$ and $p=0.0038$). The fact that even Crohn's colitis has higher levels of serum α-1AP than ulcerative colitis suggests that these differences are a disease phenomenon other than due to the part of the bowel affected.

SECTION 11B

WGLF α-1 ACID GLYCOPROTEIN LEVELS IN CONTROLS AND PATIENTS WITH IBD

Whole gut lavage fluid was obtained from 21 controls, 28 Crohn's disease and 23 ulcerative colitis patients and assayed for α-1 acid glycoprotein by immunoturbidimetry (see chapter 6A).

Controls gave a range of 1.80-7.20ug/ml with a median of 3.20ug/ml.

Patients with Crohn's disease gave a range of 1.20-72.80ug/ml with a median of 23.65ug/ml, significantly higher than the value for the control group ($p=0.00001$).

Patients with ulcerative colitis gave a range of 4.0-72.80ug/ml with a median of 23.20ug/ml, significantly higher than for the control group ($p=0.00001$) [Table 11B:1A and Graph 11B:1].

A level of 6.3ug/ml was two standard deviations above the median and considered high for further evaluations. The frequency of high levels of WGLF α-1 acid glycoprotein as determined by this value was analysed according to disease group (Table 11B:1B). Out of 21 controls, 2 had high levels of α-1AP in WGLF as compared to 24 out of 28 Crohn's disease patients or 16 out of 23 patients with ulcerative colitis. As stated above both these groups showed higher levels than those for controls.

DISEASE ACTIVITY AND WGLF α-1 ACID GLYCOPROTEIN

The patients with IBD were further subdivided according to disease activity based on clinical assessment and the levels of total IgG.

Active Crohn's disease patients gave a range of 6.6-72.80ug/ml with a median of 25.65ug/ml, significantly higher than controls (p=0.00001). Patients with active ulcerative colitis gave a range of 5.2-72.80ug/ml with a median of 29.20ug/ml, significantly higher than controls (p=0.00001) [Table 11B:1C, Graph 11B:1].

Inactive Crohn's disease gave a range of 1.2-72.8ug/ml with a median of 14.65ug/ml, not significantly higher than controls (p=0.0993). Inactive UC gave a range of 4.0-67.50ug/ml with a median of 30.86ug/ml, significantly higher than controls (p=0.0005) [Table 11B:1D].

COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN BETWEEN DISEASE GROUPS

Levels of α-1 acid glycoprotein were compared between disease groups.

There were no significant difference in the levels of WGLF α-1 acid glycoprotein between disease groups regardless of disease activity (Table 11B:1E).

COMMENT

The source of α-1 acid glycoprotein in WGLF is probably serum, due to a leak of serum protein into the intestinal lumen. This leakage would be expected to be greater when the mucosal barrier is damaged as in inflammatory bowel disease. Another source of WGLF α-1AP could be from the bile flowing from the liver directly into the intestinal lumen. Whereas both inactive Crohn's disease and inactive ulcerative colitis had significantly higher levels of α-1AP than controls in serum only, inactive ulcerative colitis had higher levels in WGLF. The possible explanation for this is that where the differences are small as between inactive disease groups and controls, difference in levels will be detected more easily if sampling occurs nearer the source of the factor being measured before dilution and diffusion occurs. Sera, which reflects directly the levels of α-1 acid glycoprotein, will be more representative.

REGIONAL INVOLVEMENT AND WGLF α-1 ACID GLYCOPROTEIN

Patients were subgrouped into regional involvement as for serum α-1 acid glycoprotein above. Patients with small bowel Crohn's disease gave a range of 1.2-72.80ug/ml with a median of 35.60ug/ml. Colonic Crohn's disease gave a range of 2.8-72.80ug/ml with a median of 18.60ug/ml, not

significantly lower than small bowel Crohn's disease (p=0.4757). Patients with ulcerative colitis gave a range of 4.0-72.40ug/ml with a median of 29.20ug/ml, again not significantly lower than small bowel Crohn's disease (p=0.6097) [Table 11B:2A]

The patients were further analysed according to the prevalence of high levels of α-1AP as described above (level above 6.3ug/ml). Eight of the 16 controls had high levels compared to 11 of the 13 patients with small bowel Crohn's disease, 8 of the 10 patients with colonic Crohn's disease or 20 of the 21 patients with ulcerative colitis (Table 11B:2B). The same analysis was done for patients with active and inactive disease, the results of the statistical analysis are described below .

Active small bowel Crohn's disease gave a range of 9.2-67.20ug/ml with a median of 39.45ug/ml. Active colonic Crohn's disease gave a range of 6.6-72.80ug/ml with a median of 29.65ug/ml, not significantly different from active small bowel Crohn's disease (p=0.6365) [Table 11B:2C]. Patients with active ulcerative colitis gave a range of 5.2-72.80ug/ml with a median of 29.20ug/ml not significantly different from small bowel Crohn's disease (p=0.5382). There was no significant difference either between the inactive disease subgroups (Table 11B:2D) or between colonic Crohn's disease and ulcerative colitis (Table 11B:2E).

COMMENT

There were no differences in the levels of α-1AP in WGLF between disease groups or regional involvement, regardless of activity. I suggest that this because only a small fraction of serum α-1AP diffuses into the intestinal secretions and that the contribution via bile is minimal.

CORRELATIONS

THE RELATIONSHIP BETWEEN SERUM AND WGLF α-1 ACID GLYCOPROTEIN

Coefficients of correlation between levels of α-1AP in sera and WGLF were calculated for each disease group and subgroup. These are shown in table 11B:3A.

There was no strong correlation between serum and lavage α-1AP in controls or patients with ulcerative colitis even when the latter were subdivided into active and inactive subgroups (Table 11B:3A).

Crohn's disease gave a strong correlation ($r=0.730$, $p=0.0001$) as did patients with active Crohn's disease ($r=0.615$ $p=0.002$). The patients with inactive Crohn's disease also gave a strong positive correlation ($r=0.917$ $p=0.0001$).

COMMENT

These findings would suggest that there is more free leakage of α-1 acid glycoprotein into the intestinal lumen in Crohn's disease than in ulcerative colitis. I suggest that this is because unlike the mucosally confined ulcerative colitis, Crohn's disease is panmural (see chapter 1A). In addition it may indicate that systemic disease parallels mucosal disease in Crohn's disease more than in ulcerative colitis so that the increased leakage of proteins into the lumen, though independent of the acute phase response, corresponds to the increase in the hepatic production of α-1 acid glycoprotein (see chapter 4).

THE RELATIONSHIP BETWEEN SERUM AND WGLF α-1 ACID GLYCOPROTEIN AND DISEASE ACTIVITY IN IBD

Coefficients of correlation between levels of α-1AP and WGLF total IgG (a measure of disease activity) were calculated for each disease group and subgroup. These are shown in table 11B:3B.

There was no strong correlation between WGLF or serum α-1AP and WGLF total IgG for all patients with ulcerative colitis even when subgrouped according to disease activity (Table 11B:3B).The same applied to patients with Crohn's disease (Table 11B:3C).

COMMENT

The lack of a relationship between WGLF or serum α-1AP and WGLF IgG could be because , the levels of the α-1AP, as an acute phase protein, are affected by many other conditions such as infections, malignancy or other inflammatory conditions like rheumatoid arthritis (see chapter 3).

THE RELATIONSHIP BETWEEN WGLF OR SERUM α-1 ACID GLYCOPROTEIN AND WGLF TUMOUR NECROSIS FACTOR

Coefficients of correlation between levels of α-1AP and WGLF tumour necrosis factor were calculated for each disease group and subgroup. These are shown in tables 11B:4A-C.

There was no strong correlation between α-1 acid glycoprotein levels either in serum or lavage and tumour necrosis factor levels in the same patients for controls as well as all patients with Crohn's disease or ulcerative colitis (Table 11:4A) even when they were further subgrouped into active disease groups (Table 11B:4B) or inactive disease groups (Table 11B:4C).

COMMENT

There was no correlation between the levels of α-1AP in

serum or WGLF and the levels of tumour necrosis factor in WGLF. This applied even when patients were subgrouped according to disease activity. I suggest that though TNF is one of the cytokines involved synergistically, with IL-1 and IL-6, in inducing hepatic synthesis of α-1AP, as discussed in chapter 4, it alone is not a strong inducer of α-1AP synthesis.

SECTION 11C

ELEMENTAL DIET AND α-1 ACID GLYCOPROTEIN

STUDY POPULATION

Eleven IBD patients were involved in this study. Serum and WGLF specimens were collected before and on day 7 after commencing elemental diet therapy. The same specimens were analysed for TNF, as TNF plays a part in the control of α-1 acid production. Table 11C is a summary of the patient data and their α-1AP levels in serum and WGLF.

RESULTS

On global clinical assessment 3 of the IBD patients improved, 4 showed no change and four deteriorated. Their results are plotted in Graph 11C.
The patient with coeliac disease had initially normal levels of serum and WGLF α-1AP that remained normal during the elemental diet.

SERUM α-1 ACID GLYCOPROTEIN AND ELEMENTAL DIET

Two of the responders (the patients who improved clinically) started with high levels of serum α-1AP which

increased despite the improvement in their clinical condition. In third responder who had an initially 'low' level of serum α-1AP, there was a slight fall during elemental diet treatment.

Three of the patients who clinically showed no change in their condition started with high serum levels of α-1 acid glycoprotein. In two of these the levels of α-1AP rose while in the third patient the serum level of α-1AP remained unchanged during elemental diet. The fourth patient started with a normal concentration of α-1 acid glycoprotein which rose while on elemental treatment.

All the four patients whose clinical condition deteriorated started with high levels of serum α-1AP and these increased during the course of treatment.

Therefore initial levels of serum α-1 acid glycoprotein did not predict the outcome of treatment with elemental diet.

WGLF α-1 ACID GLYCOPROTEIN AND ELEMENTAL DIET

Three patients improved clinically (responders). Two of the patients who responded started with high levels of lavage α-1AP and the levels fell subsequently while on elemental diet (BS and DM). These are the same patients who had high levels in serum which rose despite improvement in their clinical condition. The level of α-1AP in WGLF of the third patient who improved was

initially low (DB) and it did not change during the course of treatment.

The four patients whose clinical condition remained unchanged started with levels of α-1AP in their WGLF similar to that of responders. In two of them the levels showed little change (DS, EM) one showed a slight rise while in one there was a slight fall.

Three of the four patients who deteriorated began with a high levels of α-1 acid glycoprotein. In two of these the levels decreased and in one there was an increase. One patient had a low initial level and this did not change during the course of ED therapy.

The only uniform result is in patients whose clinical condition remained unchanged: their levels of α-1AP also did not change during the course of the treatment.

The patient with coeliac disease had already been on a gluten free diet and her levels α-1 acid glycoprotein were low and remained low during the whole study. She did not show clinical improvement on this treatment.

COMMENT

These results show that there is no relationship between a response or lack of response to elemental diet and α-1AP levels whether in lavage or serum. The number of patients on elemental diet is small (11). This is because patients were prescribed elemental diet only when other

modalities of treatment had failed. Perhaps if more numbers of a patients were studied their may be a relationship. This is however more unlikely as α-1AP being an acute phase protein is subject to many factors such as infections and other connective tissue disease (see chapter 4).

GENERAL COMMENT

The aim of these experiments was to establish the pattern of α-1 acid glycoprotein levels in controls and IBD patients. The levels in serum and WGLF were significantly higher in patients than in controls for both active and inactive IBD groups except for inactive Crohn's disease WGLF α-1 acid glycoprotein. Further breakdown showed that the level of significance compared to controls was higher in patients with active disease. There was no difference between active and inactive IBD probably reflecting the insensitivity of this acute phase protein or suggesting that there is usually underlying active disease in patients who may be clinically classified as inactive. The same applied to the levels in WGLF which largely reflect leakage from serum (discussed above chapter 11B). The contribution from the liver via the bile duct is therefore minimal.

Comparisons between Crohn's disease patients with small bowel and those with large bowel disease showed that the

patients with Crohn's disease have higher serum levels of α-1 acid glycoprotein than ulcerative colitis patients regardless of the region involved.

There was no relationship between disease response to elemental diet and α-1 acid glycoprotein levels both in lavage and serum. This lack of relationship could be probably because α-1 acid glycoprotein is a more general index of disease activity rather than specific to inflammatory bowel disease.

Despite the fact that α-1 acid glycoprotein was found to be a predictor of relapse in IBD (Wright et al., 1987), for the patients studied there was no relationship to disease response with elemental diet therapy.

APPENDIX FOR CHAPTER 11

TABLE 11:1A STUDY POPULATION

	CROHN'S DISEASE	ULCERATIVE COLITIS	CONTROLS
NUMBERS	28	23	21
SEX M:F	13:15	9:14	7:13
AGE			
MEDIAN	39	37	47
RANGE	13-82	22-78	20-79

DISEASE DISTRIBUTION

CROHN'S DISEASE		ULCERATIVE COLITIS	
Small bowel	13	Rectum only	7
Ileocolonic	5	Left sided	4
Colonic	6	Pancolitis	10
Rectal	2	Pouchitis	1
Perianal	1	Microscopic	1
Anal	1	13 Active and 10 inactive	

18 Active and 10 inactive

5 resections

TABLE 11:1A CONTINUED

CONTROLS	
Polyps	5
Constipation	4
Healthy controls	3
Diarrhoea-NAD	1
Duodenal ulcer	1
Colonic angiodysplasia	1
NAD	5
Megacolon	1

DRUG TREATMENT

CROHN'S DISEASE		ULCERATIVE COLITIS	
No drugs	10	No drugs	7
Prednisolone only (Pred)	4	Pred, ASA and CFM	3
Sulphasalazine (SLZ)	7	ASA and CFM	2
5-Amino Salicylates only (ASA)	2	ASA only	4
SLZ and Pred	3	ASA and PRED	2
Pred, ASA and Colifoam (cfm)	0	ASA,CFM and Azath	1
SLZ, Pred and CFM	0	ASA,Pred and Cyclo	1
Pred and CFM	1	CFM only	1
Colifoam only	1	Pred only	2

TABLE 11A:1B SERUM α-1 ACID GLYCOPROTEIN (mg/ml) IN CONTROLS AND PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	16	0.43	0.40 (0.19-0.73)	
CRO	26	4.11	3.90 (0.05-7.50)	0.00001
UC	21	1.90	1.60 (0.42-5.40)	0.004

p = Probability that the difference is a chance finding

TABLE 11A:1C THE PREVALENCE OF HIGH LEVELS OF SERUM α-1 ACID GLYCOPROTEIN IN CONTROLS, CROHN'S DISEASE AND ULCERATIVE COLITIS. Levels above 0.69mg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>0.69	N	>0.69	N	>0.69
CONTROL	(16)	1		NA		NA
CD	(26)	25***	(17)	16***	(9)	9
UC	(21)	20***	(12)	11*	(9)	9

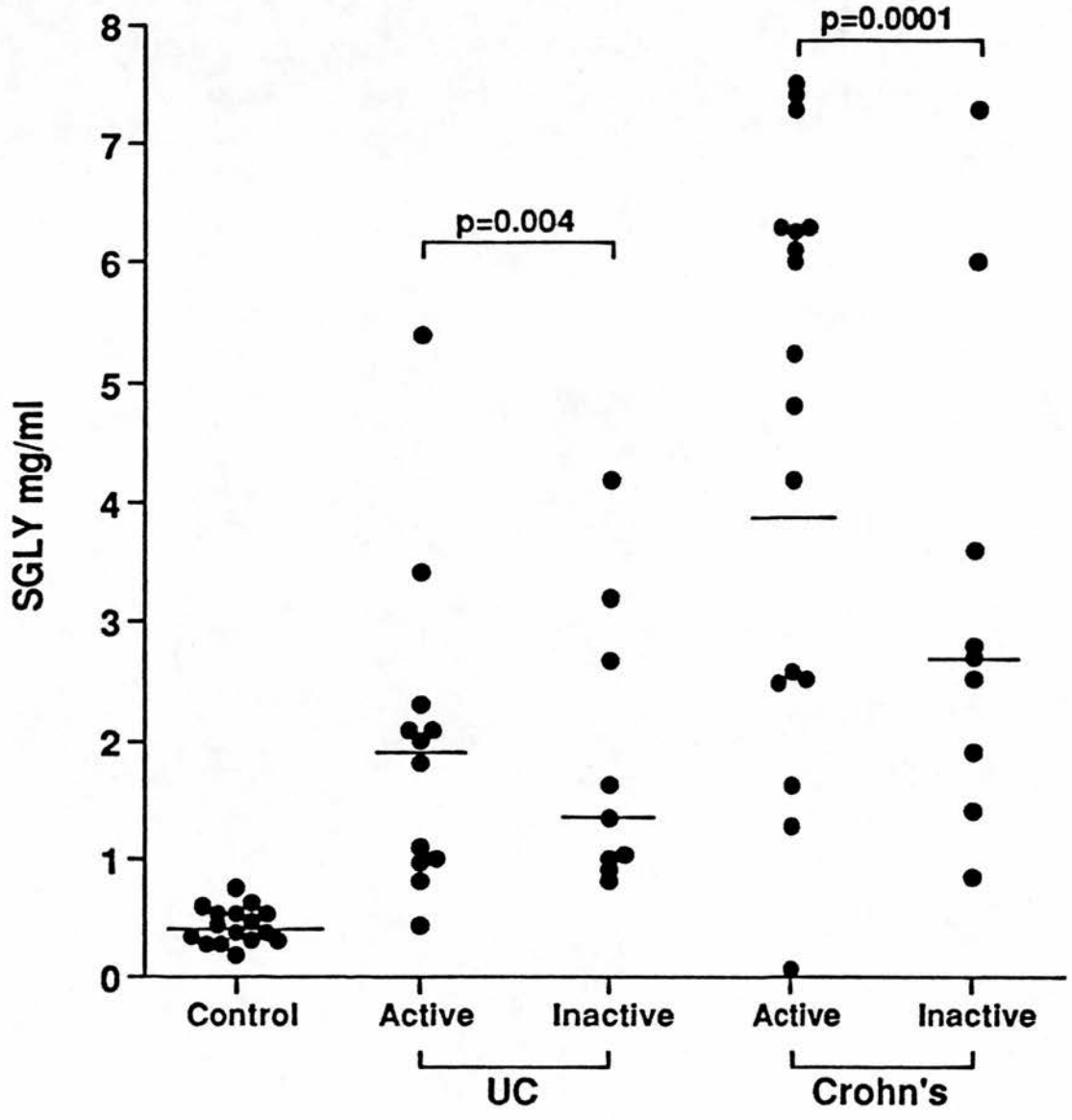
NA = Not applicable

* p < 0.05 ** p < 0.01 *** p < 0.005

N = Number of subjects in the group

Graph 11A:1

SERUM α-1 ACID GLYCOPROTEIN IN CONTROLS, CROHN'S DISEASE AND ULCERATIVE COLITIS



On the x axis are the subject groups plotted against their levels of serum α-1 acid glycoprotein on the y axis. All subgroups, active and inactive, had significantly higher levels than controls.

TABLE 11A:1D SERUM α-1 ACID GLYCOPROTEIN (mg/ml) IN CONTROLS AND PATIENTS WITH ACTIVE CROHN'S DISEASE AND ACTIVE ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	16	0.43	0.40 (0.19-0.73)	
aCRO	17	4.58	5.25 (0.05-7.50)	0.00001
aUC	12	1.94	1.90 (0.42-5.40)	0.00001

TABLE 11A:1E SERUM α-1 ACID GLYCOPROTEIN (mg/ml) IN CONTROLS AND PATIENTS WITH INACTIVE CROHN'S DISEASE AND INACTIVE ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	16	0.43	0.40 (0.19-0.73)	
inaCRO	9	3.22	2.70 (0.85-7.30)	0.0001
inaUC	9	1.86	1.32 (0.80-4.20)	0.029

p = Probability that the difference is a chance finding

TABLE 11A:1F COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN LEVELS BETWEEN CROHN'S DISEASE AND ULCERATIVE COLITIS PATIENTS.

DISEASE GROUPS	p Value
CRO vs UC	0.3751
aCRO vs inaCRO	0.2053
inaCRO vs inaUC	0.1333
aCRO vs aUC	0.0032
inaUC vs AUC	0.8870

p = Probability that the difference is a chance finding

TABLE 11A:2A A COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN (mg/ml) LEVELS BETWEEN SMALL BOWEL (SB) CROHN'S DISEASE AND COLONIC (CN) CROHN'S DISEASE OR ULCERATIVE COLITIS (UC).

GROUP	N	MEAN	MEDIAN (RANGE)	p
CRO (SB)	11	4.58	5.25 (0.05-7.50)	
CRO (CN)	10	4.49	5.10 (1.28-7.40)	1.0000
UC	21	1.90	1.60 (0.42-5.40)	0.0079

TABLE 11A:2B THE PREVALENCE OF HIGH LEVELS OF SERUM α-1 ACID GLYCOPROTEIN IN SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). Levels above 0.69mg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>0.69	N	>0.69	N	>0.69
CONTROL	(16)	1		NA		NA
SB	(11)	10	(6)	6	(5)	4
CN	(10)	10	(8)	8	(2)	2
UC	(21)	20**	(12)	11*	(9)	9

* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

TABLE 11A:2C A COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN LEVELS (mg/ml) BETWEEN ACTIVE SMALL BOWEL CROHN'S DISEASE, ACTIVE COLONIC CROHN'S AND ACTIVE ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CRO (SB)	7	4.66	5.25 (0.05-7.50	
CRO (COL)	8	5.14	6.05 (1.28-7.40	0.8170
UC	12	1.94	1.90 (0.42-5.40)	0.0312

p = Probability that the difference is a chance finding

TABLE 11A:2D A COMPARISON OF SERUM α-1 ACID GLYCOPROTEIN LEVELS BETWEEN COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS. Statistical comparisons by Mann-Whitney (p values)

	p
CRO(COL) vs UC	0.0031*
aCRO(COL) vs aUC	0.0038*
inaCRO(COL) vs inaUC	0.2861

p = Probability that the difference is a chance finding

* = significant difference at 5% level

TABLE 11B:1A THE LEVELS OF α-1 ACID GLYCOPROTEIN (ug/ml) IN WGLF OF CONTROLS AND PATIENTS WITH CROHN'S DISEASE AND ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	21	3.8	3.20 (1.80-7.20)	
CRO	28	30.96	23.65 (1.20-72.80)	0.00001
UC	23	31.31	23.20 (4.00-72.80)	0.00001

TABLE 11B:1B THE PREVALENCE OF HIGH LEVELS OF WGLF α-1 ACID GLYCOPROTEIN IN CONTROLS, CROHN'S DISEASE AND ULCERATIVE COLITIS. Levels above 6.3ug/ml.

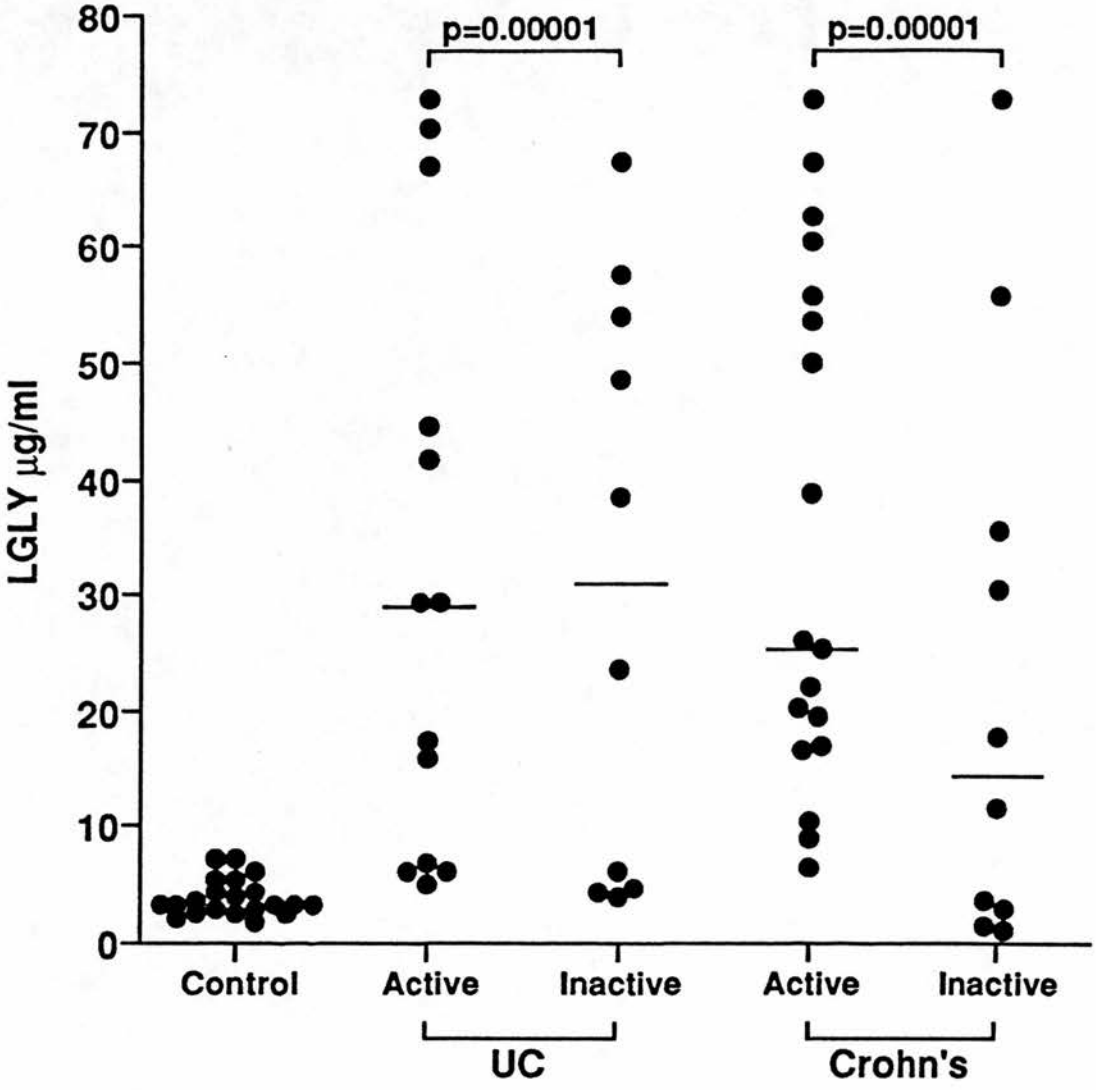
	ALL		ACTIVE		INACTIVE	
	N	>6.3	N	>6.3	N	>6.3
CONTROL	(21)	2		NA		NA
CD	(28)	24***	(18)	18***	(10)	6
UC	(23)	16***	(13)	10***	(10)	6***

NA = Not applicable

* p < 0.05 ** p < 0.01 *** p < 0.005

Graph 11B:1

WGLF α-1 ACID GLYCOPROTEIN IN CONTROLS, CROHN'S DISEASE AND ULCERATIVE COLITIS



On the x axis are the subject groups plotted against their levels of WGLF α-1 acid glycoprotein on the y axis.

TABLE 11B:1C THE LEVELS OF α-1 ACID GLYCOPROTEIN (ug/ml) IN WGLF OF CONTROLS AND PATIENTS WITH ACTIVE CROHN'S DISEASE AND ACTIVE ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	21	3.8	3.20 (1.80-7.20)	
aCRO	18	35.24	25.65 (6.60-72.80)	0.00001
aUC	13	31.65	29.20 (5.20-72.80)	0.00001

TABLE 11B:1D THE LEVELS OF α-1 ACID GLYCOPROTEIN (ug/ml) IN WGLF OF CONTROLS AND PATIENTS WITH INACTIVE CROHN'S DISEASE AND INACTIVE ULCERATIVE COLITIS.

GROUP	N	MEAN	MEDIAN (RANGE)	p
CONTROL	21	3.8	3.20 (1.80-7.20)	
inaCRO	10	23.26	14.65 (1.20-72.80)	0.0993
inaUC	10	30.86	30.95 (4.00-67.50)	0.0005*

p = Probability that the difference is a chance finding

TABLE 11B:1E COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN LEVELS BETWEEN CROHN'S DISEASE AND ULCERATIVE COLITIS.

Statistical comparisons by Mann-Whitney (p values)

DISEASE GROUPS	p Value
CRO vs UC	0.9773
aCRO vs inaCRO	0.1436
inaCRO vs inaUC	0.3075
aCRO vs aUC	0.5482
inaUC vs aUC	0.6642

p = Probability that the difference is a chance finding

TABLE 11B:2A A COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN LEVELS (ug/ml) BETWEEN SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC)

GROUP	N	MEAN	MEDIAN (RANGE)	p
CRO (SB)	13	36.74	35.60 (1.20-72.80)	
CRO (CN)	10	28.41	18.60 (2.80-72.80)	0.4757
UC	23	31.31	29.20 (4.00-72.80)	0.6097

TABLE 11B:2B THE PREVALENCE OF HIGH LEVELS OF WGLF α-1 ACID GLYCOPROTEIN IN SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). Levels above 6.3ug/ml.

	ALL		ACTIVE		INACTIVE	
	N	>6.3	N	>6.3	N	>6.3
CONTROL	(16)	8		NA		NA
SB	(13)	11	(8)	8	(5)	3
CN	(10)	8	(8)	8	(2)	0
UC	(21)	20	(12)	11	(9)	9

NA = Not applicable

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

TABLE 11B:2C A COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN LEVELS (ug/ml) BETWEEN ACTIVE SMALL BOWEL (SB) CROHN'S DISEASE, ACTIVE COLONIC (CN) CROHN'S AND ACTIVE ULCERATIVE COLITIS (UC).

GROUP	N	MEAN	MEDIAN (RANGE)	p
CRO (SB)	8	38.89	39.45 (9.20-67.20)	
CRO (COL)	8	34.71	29.65 (6.60-72.80)	0.6365
UC	13	31.65	29.20 (5.20-72.80)	0.5382

TABLE 11B:2D A COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN LEVELS (ug/ml) BETWEEN INACTIVE SMALL BOWEL (SB) CROHN'S DISEASE AND INACTIVE ULCERATIVE COLITIS (UC).

GROUP	N	MEAN	MEDIAN (RANGE)	p
CRO (SB)	5	33.30	35.60 (1.20-72.80)	
UC	10	30.86	30.95 (4.00-67.50)	0.8542

p = Probability that the difference is a chance finding

TABLE 11B:2E A COMPARISON OF WGLF α-1 ACID GLYCOPROTEIN LEVELS BETWEEN COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS. Statistical comparisons by Mann-Whitney (p values)

DISEASE GROUPS	p values
CRO(CN) vs UC	0.7540
aCRO(CN) vs aUC	0.6122

p = Probability that the difference is a chance finding

CORRELATIONS

TABLE 11B:3A CORRELATION BETWEEN SERUM AND WGLF α-1 ACID GLYCOPROTEIN LEVELS.

GROUP	N	r	p
CONTROL	16	-0.186	0.323
UC	21	-0.039	0.608
aUC	12	0.409	0.157
inaUC	9	-0.482	0.364
CRO	26	0.730	0.0001
aCRO	17	0.615	0.002
inaCRO	9	0.917	0.0001

p = Probability that the correlation (r) is due to chance

TABLE 11B:3B CORRELATIONS BETWEEN WGLF (LGLY) OR SERUM (SGLY) α-1 ACID GLYCOPROTEIN AND WGLF TOTAL IGG (LIGG) IN CONTROLS AND PATIENTS WITH ULCERATIVE COLITIS.

	N	LGLY vs LIGG		N	SGLY vs LIGG	
		r	p		r	p
CONTROL	21	-0.107	0.776	16	0.301	0.514
UC	23	-0.027	0.467	21	-0.144	0.551
aUC	13	-0.337	0.315	12	-0.186	0.434
inaUC	10	0.298	0.363	9	-0.543	0.244

p = Probability that the correlation (r) is due to chance

TABLE 11B:3C CORRELATIONS BETWEEN WGLF (LGLY) OR SERUM (SGLY) α-1 ACID GLYCOPROTEIN AND WGLF TOTAL IGG (LIGG) IN CONTROLS AND PATIENTS WITH CROHN'S DISEASE.

	N	LGLY vs LIGG		N	SGLY vs LIGG	
		r	p		r	p
CRO	28	0.204	0.752	26	0.067	0.513
aCRO	18	-0.270	0.684	17	-0.348	0.121
inaCRO	10	0.488	0.666	9	0.275	0.768

p = Probability that the correlation (r) is due to chance

TABLE 11B:4A CORRELATIONS BETWEEN WGLF α-1 ACID GLYCOPROTEIN (LGLY) OR SERUM α-1 ACID GLYCOPROTEIN (SGLY) AND WGLF TUMOUR NECROSIS FACTOR.

GROUP	LGLY			SGLY		
	N	r	p	N	r	p
CONTROL	15	0.125	0.586	15	-0.033	0.743
CRO	26	-0.048	0.480	23	-0.018	0.143
UC	21	0.178	0.816	23	-0.158	0.127

TABLE 11B:4B CORRELATIONS BETWEEN WGLF α-1 ACID GLYCOPROTEIN (LGLY) OR SERUM α-1 ACID GLYCOPROTEIN (SGLY) AND WGLF TUMOUR NECROSIS FACTOR IN ACTIVE IBD.

GROUP	LGLY			SGLY		
	N	r	p	N	r	p
aCRO	17	0.041	0.337	17	0.045	0.559
aUC	12	0.279	0.725	12	-0.511	0.087

TABLE 11B:4C CORRELATIONS BETWEEN WGLF α-1 ACID GLYCOPROTEIN (LGLY) OR SERUM α-1 ACID GLYCOPROTEIN (SGLY) AND WGLF TUMOUR NECROSIS FACTOR IN INACTIVE IBD.

GROUP	LGLY			SGLY		
	N	r	p	N	r	p
inaCRO	9	-0.259	0.407	10	-0.044	0.310
inaUC	9	-0.031	0.338	10	-0.303	0.964

p = Probability that the correlation (r) is due to chance

TABLE 11C

ALPHA-1 ACID GLYCOPROTEIN AND ELEMENTAL DIET

						CLINICAL DATA																
NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	TP	ALB	LGLY	SGLY	HB	CRP	ESR	WT	TP	ALB	LGLY	SGLY	CO
DM	29	F	1	STEROID FAILURE	NONE	21	13	2.3	37	50	71	36	9.2	2.48	13	1.5	15	49	71	38	4.2	3.44	*
BS	28	M	2	STEROID FAILURE	NONE	10	11	10	90	46	64	34	29.2	2.1	11	1.5	50	51	64	34	14	3.1	*
DS	30	F	1	RECCURRENT ACTIVE DISEASE	HEMICOLON	31	10	3.6	25	42	51	30	22.2	0.1	11	3.7	32	44	59	31	22	1.2	N
DB	19	M	1ILC	RECURRENT ACTIVE DISEASE	ILEUM + COLON	21	10	5.5	63	52	79	36	1.2	0.7	10	1.5	37	52	76	34	1.6	0.7	*
AM	38	F	1	SB OBSTRUCTION CROHN'S DISEASE	TOTAL COLON	21	13	3.6	34	68	69	36	26	1.6	15	1.5	17	68	76	42	31	1.6	D
EW	25	F	1COL	RECURRENT ACTIVE DISEASE	NONE	14	11	2	83	45	64	34	17	13	10	3.9	80	15	64	34	20	15	D
MI	50	F	1	ACTIVE ILEAL DISEASE	RIGHT COLON	21	14	1.5	–	64	31	6.6	2.5	13	1.5	11	–	57	30	1	3.4	2.8	D
JM	54	F	1COL	RECURRENT ACTIVE DISEASE	SIGMOID	14	10	10	46	66	65	32	20	2.6	10	1.5	42	64	66	33	5.4	2.76	D
CC	16	M	1COL	ACTIVE CROHN'S DISEASE	SUBTOTAL COLECTOMY	14	10	7	25	25	74	28	49	3.1	15	1.5	33	26	78	31	56	4	D
SJ	26	M	1COL	ABD. PAIN AND DIARRHOEA	NONE	21	14	1.5	33	82	82	40	2.8	2.5	14	1.5	13	83	78	40	3	2.8	D
EM	51	F	1	SMALL BOWEL OBSTRUCTION	TERMINAL ILEUM	14	13	11	79	57	73	40	11	1.9	12	16	92	60	73	40	9.6	2.2	N
ES	34	F	3	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	80	43	4	0.7	12	1.5	10	41	72	44	2	0.7	N

**←BEFORE ELEMENTAL DIET ⟶ *←ON ELEMENTAL DIET ⟶ *

ABBREVIATIONS

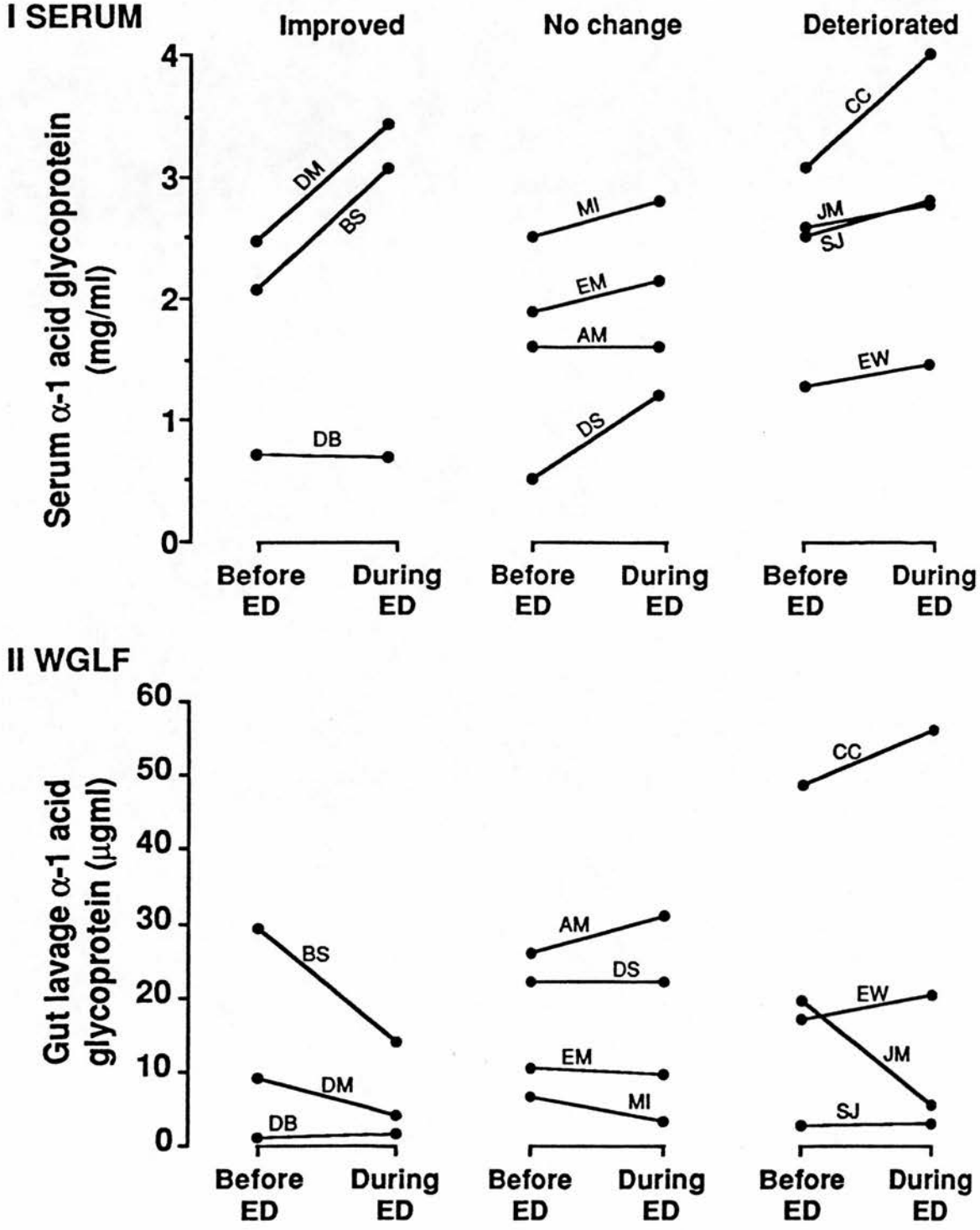
1 = ILEAL CROHN'S DISEASE
2 = ULCERATIVE COLITIS
3 = COELIAC DISEASE
COL = colonic Crohn's disease
ILC = ileocolonic Crohn's disease

CLINICAL OUTCOME (CO)

* = IMPROVED
D = DETERIORATED
N = NO CHANGE
LGLY = WGLF ALPHA-1 ACID GLYCOPROTEIN
SGLY = SERUM ALPHA-1 ACID GLYCOPROTEIN
DOD = DAYS ON DIET

Graph 11C

SERUM α-1 ACID GLYCPROTEIN AND WGLF α-ACID GLYCOPROTEIN AND ELEMENTAL DIET



Levels of α-1 acid glycoprotein in serum (top) and WGLF (bottom) before and during elemental diet. Initial levels of α-1 acid glycoprotein did not predict outcome.

CHAPTER TWELVE:

SOLUBLE INTERLEUKIN-2 RECEPTOR

INTRODUCTION

There is growing evidence that T cell function is of primary importance in the immunopathogenesis of IBD (Choy et al., 1990; Mueller et al., 1990). Interleukin-2 and its receptors are pivotal in the immune function of T cells (Smith and Cantrell, 1985). The density of the inducible IL-2 receptor on the cell membranes determines whether the immune response progresses or not (Wang and Smith, 1987; chapter 4A). Soluble interleukin 2 receptor (sIL-2R), a shed variety of the p55 molecule has been found to be increased in the serum of patients with active IBD patients. There is little literature describing intestinal T cell activity in IBD in vivo despite the acknowledged fact that the intestinal mucosa is an area of intense immune activity (reviewed in chapter 1).

THE AIM OF THIS STUDY

The primary aim of this study was to evaluate systemic (serum) and intestinal (WGLF) T cell activity by measuring levels of sIL-2R in controls and disease groups. Secondly, having established the pattern to study T cell activity in patients who had been prescribed elemental diet for clinical reasons. My hypothesis was that elemental diet works by influencing the immune

system. Results presented in chapter 10 in this thesis showed that improvement with elemental diet was not via the humoral arm of the immune response. If, indeed immune modulation is the mechanism by which elemental diets act, then it would be expected that patients who responded to elemental diet would show a down-regulation of their T cell activity.

SUBJECTS AND PROTOCOL

A total of 69 IBD patients (45 Crohn's disease and 24 ulcerative colitis), 8 gluten enteropathy patients and 20 controls were studied (Table 12:1).

Of the 45 patients with Crohn's disease the macroscopic disease at the time of lavage was as follows: small bowel (15) (4 jejunal and 11 terminal ileum), ileocolonic (13), colonic (10), rectal (5) (one associated with anal disease), perianal (1) and one with microscopic disease. Ten had undergone resections. All the patients were on normal diet. As regards drug treatment 10 patients were on steroids the dose ranging between 5-40mg per day, 13 on sulphasalazine, 7 on sulphasalazine and steroids, 1 on prednisolone, mesalazine and azathioprine, 1 on sulphasalazine, prednisolone and colifoam enemas, 1 on prednisolone and colifoam enemas and 12 on no drug therapy for their Crohn's disease. The age range was 14-83 with a median of 44. Twenty-eight had active disease

and in 17 the disease was inactive as assessed clinically and also based on WGLF total IgG (see chapter 7).

None of 24 patients with ulcerative colitis had had any resections. The macroscopic distribution of the disease at time of sampling was as follows: 11 pancolitis, 3 left-sided involvement, 8 proctitis, 1 UC pouchitis and 1 with microscopic ulcerative colitis. Seven were on no drug treatment, 3 were on oral prednisolone and an amino-salicylate (ASA) type drug plus rectal steroids, 2 were on an ASA group drug plus rectal steroids, 1 was on rectal steroids, 6 on an oral ASA group only, 3 were taking oral prednisolone as well as ASA, 1 was on a combination of colifoam enemas, azathioprine and ASA and 1 was on cyclosporin in addition to oral steroids and ASA. The age range for this group was 22-79 with a median of 38. Of the 24, 14 had active disease at time of study. The age range for the 8 patients with gluten related enteropathy was 19-68 with a median of 41. Seven had coeliac disease and these were all on a gluten-free diet. One had dermatitis herpetiformis and was on a normal diet.

Most of the controls had presented with symptoms that required gastro-intestinal investigations but on final clinical assessment the diagnosis was non-inflammatory bowel conditions. The final diagnoses in the controls were as follows: non-inflammatory polyps in the colon (2), constipation (5), diarrhoea with no abnormality

detected (1), duodenal ulcer (1), unexplained abdominal pain (8) and 3 controls. The age range was 21-85 with a median of 46.

All controls were on normal diet.

Serum and WGLF sIL-2R was measured by ELISA as described in chapter 6.

The serum was drawn on the day of gut lavage collection so that the two specimens reflected systemic and mucosal immune activity at corresponding times.

Comparisons in the levels sIL-2R in serum (BIL2R) and WGLF (LIL2R) were made between controls and the IBD patients. Comparisons were also made when the patients with IBD were further subgrouped according to whether they had active or inactive disease and according to the regional distribution of disease.

Correlations analyses between the levels of sIL-2R in sera and gut lavage fluid were performed to define their relationship if any.

Finally levels of BIL2R and LIL2R were measured in patients who were prescribed elemental diet before and after a period of elemental diet.

SECTION 12A

SERUM sIL-2R (BIL2R) LEVELS IN CONTROLS AND IBD PATIENTS

A total of 11 specimens of serum were collected from

controls, 20 from patients with ulcerative colitis and 38 from patients with Crohn's disease and assayed for sIL-2R.

The controls gave a range of 171-544U/ml with a median of 235U/ml.

The range for patients with ulcerative colitis (UC) was 50-1986U/ml with a median of 461U/ml, this was significantly higher than the levels for the controls ($p=0.039$). Crohn's disease (CD) patients gave a range of 10-2844U/ml with a median of 610U/ml, again this was significantly higher than controls ($p=0.0045$) (Table 12A:1B and Graph 12A:1).

On further analysis of levels in controls, values of more than 510U/ml were 2 standard deviations above the mean, and were classified as high when studying the frequency of high levels in disease groups. Only one of the 11 controls had high levels of serum sIL-2R compared to 9 out of 20 patients with ulcerative colitis or 25 out of 38 patients with Crohn's disease (Table 12A:1C). These results were significantly higher than the levels for the controls as shown above (Table 12A:1B). Similarly the prevalence of high levels of serum sIL-2R in patients with active and inactive diseases was analysed as shown in table 12:1C; the results of the statistical analysis are shown and discussed below.

DISEASE ACTIVITY AND SERUM sIL-2R

Patients were further subdivided according to whether they had active disease or inactive disease based on clinical assessment and WGLF total IgG (chapter 7). Both patients with active Crohn's disease ($p=0.0016$) and those with active ulcerative colitis ($p=0.0151$), had significantly higher levels of sIL-2R in the serum than controls (Table 12A:1C, Table 12A:1D and Graph 12A:1). Conversely, both inactive CD ($p=0.1320$) and inactive UC ($p=0.4090$) did not have significantly higher levels than controls (Table 12A:1C, Table 12A:1E and Graph 12A:1).

A COMPARISON OF SERUM sIL-2R BETWEEN DISEASE GROUPS

There was no significant difference in the serum sIL-2R concentrations between UC and CD ($p=0.3904$), active CD and inactive CD ($p=0.0526$), or between active UC and inactive UC ($p=0.1535$). There was no significant difference between active CD and active UC ($p=0.5702$) nor between inactive CD and inactive UC ($p=0.6904$) [Table 12A:1F].

COMMENTS

These results show that serum sIL-2R is raised in patients with active IBD. This is in agreement with other reports (Brynskov and Tvede, 1990; Mahida et al., 1990). There was no significant difference in serum sIL-2R levels between patients with ulcerative colitis and those with Crohn's disease. There was no significant difference between the serum levels of sIL-2R of patients with active IBD compared to the with inactive IBD. Though the levels of statistical significance were not reached (perhaps with more numbers these would be reached) the trend for serum levels of sIL-2R as shown by the means, median and difference from controls (Tables 12A:1D and 12A:1E) is that the levels were higher in patients with active IBD than the levels for patients with inactive IBD. Similarly the mean and median for the levels of serum sIL-2R were higher in patients with inactive IBD than in controls (Table 12A:1E).

The patients with inactive IBD therefore had intermediate values between active disease and controls. This suggests that patients who are clinically classified as having inactive disease still have some underlying disease activity for which we do not have clinical means sensitive enough to monitor.

Thus there is always a background of upregulated immune activity, as measured by serum sIL-2R levels, despite the

lack of overt clinical manifestations. On the otherhand the lack of significant differences in the levels of serum sIL-2R between controls and inactive disease groups means that, with disease remission, there is down-regulation of immune activity towards normal in the inactive groups.

REGIONAL INVOLVEMENT AND SERUM sIL-2R IN CROHN'S DISEASE

Crohn's disease patients were further subdivided according to the distribution of disease into four groups; small bowel (mouth to ileum with no colonic involvement), ileocolonic, colonic (no small bowel involvement) and Crohn's proctitis.

Patients with small bowel (SB) Crohn's disease had significantly higher levels of serum sIL-2R than controls range 63-1484U/ml with a median of 664U/ml ($p=0.015$). The patients with colonic (CN) Crohn's disease gave a range of 304-2845U/ml with a median of 1077U/ml, again this was significantly higher than for controls ($p=0.0014$). There was no significant difference between either ileocolonic (ILC) Crohn's disease ($p=0.1106$) or Crohn's proctitis (PR) ($p=0.0.0786$) and controls (Table 12A:2A and Graph 12A:2).

The prevalence of high levels of sIL-2R in serum (>510U/ml) were analysed according to regional involvement of Crohn's disease. As table 12A:2B shows

only 1 out 11 controls had high levels of serum sIL-2R compared to 9 out of 13 patients with small bowel Crohn's disease and 7 out of 9 patients with colonic Crohn's disease. The same was applied for patients with active and inactive disease. The results of the statistical evaluations are discussed below.

LEVELS OF SERUM sIL-2R IN SERUM OF PATENTS WITH CROHN'S DISEASE ACCORDING TO DISEASE ACTIVITY AND REGIONAL INVOLVEMENT

Patients with Crohn's disease were further subgrouped according to regional involvement of disease and disease activity based on clinical assessment and WGLF total IgG. Their sera were assayed for sIL-2R and the results compared with those of controls (Table 12A:2C).

Patients with active small bowel disease had significantly higher levels than controls with a range of 63-1484U/ml and a median of 638U/ml ($p=0.0186$), as did the patients with active colonic Crohn's disease ($p=0.0021$) and active Crohn's proctitis ($p=0.0223$). The seven patients with active ileocolonic disease patients gave high levels median 609U/ml and range of 80-1181U/ml but not significantly different from controls (Table 12A:2C).

There was no significant difference between controls and any of the subgroups with inactive disease (Table 12A:2B

and Table 12A:2D).

COMMENTS

These results show that the levels of serum sIL-2R were significantly higher than controls in all active disease subgroups except for the patients with ileocolonic Crohn's disease. This is partly explained by the fact that 7 out of these 9 ileocolonic patients had had resections so that their area of disease activity would be less than with an intact gastro-intestinal tract. Since serum sIL-2R may be derived predominantly from the gastro-intestinal tract, as discussed in chapter 4A, it would reflect only a fraction of the true levels in the intestines and the difference from controls would not be apparent. However as WGLF sIL-2R reflects local activity and is of local origin it would be more likely to show true similarities or differences for intestinal diseases. A similar analysis was done for levels of sIL-2R in WGLF of these groups (see below).

LEVELS OF SERUM sIL-2R IN ULCERATIVE COLITIS ACCORDING TO REGIONAL INVOLVEMENT

Ulcerative colitis patients were further subdivided according to the distribution of disease into three groups; pancolitis (involving the whole colon), left

sided (LS) and ulcerative proctitis (PR).

Patients with pancolitis had significantly higher levels of serum sIL-2R levels than controls range 209-1800U/ml with a median of 544U/ml (p=0.0265). The patients with ulcerative proctitis gave a range of 236-1986U/ml with a median of 493U/ml again this was significantly higher than the levels for the controls (p=0.0444) [Table 12A:3A and Graph 12A:3).

Table 12A:3B shows the prevalence of high levels of serum sIL-2R in ulcerative colitis patients according to regional involvement of disease. Out of 10 patients with pancolitis, 5 had high levels and out of 6 patients with ulcerative colitis 3 had high levels these differences were significant compared to controls who had only 1 high levels out of 11 (Table 12A:3A). The analysis was also done for patient with active and inactive ulcerative colitis in the subgroups. The statistical evaluations of these are discussed below.

There were only 3 patients with left sided disease from whom two results were obtained no statistical analyses were carried out. This group therefore is not considered any further.

REGIONAL INVOLVEMENT, DISEASE ACTIVITY AND SERUM sIL-2R IN ULCERATIVE COLITIS

Patients with active pancolitis had significantly higher levels of serum sIL-2R than controls with a range of 249-1800U/ml and a median of 669U/ml (p=0.005), but not the three patients with active proctitis (p=0.0617) though all had high values (367, 583, and 1986U/ml). The lack of significant difference from controls for the ulcerative proctitis group could be due to the small numbers studied (Table 12A:3B and Table 12A:3C).

The numbers of patients with inactive ulcerative colitis were small; 3 with inactive pancolitis and 3 with inactive ulcerative proctitis. Statistical analysis were not performed for these groups.

SECTION 12B

THE LEVELS OF WGLF sIL-2R IN CONTROLS AND IBD

Whole gut lavage fluid specimens were collected from 20 controls, 24 patients with ulcerative colitis and 45 with Crohn's disease.

In whole gut lavage fluid from controls sIL-2R was largely undetectable (<10U/ml) with a range of <10-97U/ml and a median of <10U/ml. Patients with ulcerative colitis gave a range of <10-149U/ml with a median of 39U/ml and this was not significantly higher than controls ($p=0.0533$). Patients with Crohn's disease gave a range of 10-350U/ml with a median of 56U/ml and this was significantly higher than the levels for controls ($p=0.0003$). The values of the ulcerative colitis group were midway between controls and Crohn's disease patients (Table 12B:1A and Graph 12B:1).

Analysis of the sIL-2R in WGLF of controls showed that 70 was 2 standard deviations above the median and this was used to denote high values for further analysis (Table 12B:1B). This showed that out of 20 controls ,2 had high levels of sIL-2R in their WGLF compared to 8 out of 24 patients with ulcerative colitis or 17 out of 45 patients with Crohn's disease. These were significantly higher than the levels for controls as discussed above. Further analysis of the frequency of high levels in active and

inactive disease were made (Table 12B:1B). The statistical results of the active and inactive subgroups are discussed below.

DISEASE ACTIVITY AND WGLF sIL-2R

Patients were further subgrouped according to disease activity and their levels of sIL-2R in WGLF compared with controls.

Both active Crohn's disease (p=0.0005) and active ulcerative colitis (p=0.0136) had significantly higher levels of sIL-2R in WGLF than controls (Table 12B:1B and Table 12B:1C and Graph 12B:1).

There was no significant difference between inactive UC and controls (p=0.6284). The inactive CD group gave a range of <10-350U/ml with a median of 45U/ml and this was significantly higher than controls (p=0.0064) [Table 12B:1D; Table 12B:1B and Graph 12B:1].

There was no significant difference in the levels of sIL-2R in WGLF between ulcerative colitis patients and Crohn's disease (p=0.0659), active CD and inactive CD (p=0.4329), active UC and inactive UC (p=0.0895) or between active CD and active UC (p=0.7387). Patients with inactive CD had higher values median 45U/ml range 10-350U/ml than inactive UC median 24U/ml range 10-73U/ml and the difference was of borderline significance (p=0.042) [see Table 12B:1E].

COMMENT

There were a few controls who had detectable levels (10 out of 20 with only 2 above 70) in their WGLF. This is probably due to the fact that most of the controls were hospital referrals with some abdominal symptoms. Levels of sIL-2R are raised with increased immune activity (see chapter 2A), though this is not specific to IBD, the fact than IBD patients had higher levels than controls who also had abdominal symptoms suggests that there is an upregulation of the immune response in IBD which is significantly higher in active disease. However as levels of sIL-2R are also raised in some controls, sIL-2R levels can not be used for diagnosing IBD.

These results though similar to those in blood, show some differences. Whereas serum sIL-2R in UC was significantly raised compared to controls ($p = 0.039$) in WGLF the difference was not significant ($p=0.0533$). However both WGLF and serum sIL-2R were significantly higher in CD than controls and the differences were more pronounced in lavage ($p=0.0003$) as compared to serum ($p=0.0016$). This is in keeping with the report by Mullin et al. (1991), of increased IL-2mRNA in intestinal mucosa of Crohn's disease but not in ulcerative colitis patients compared to controls. The levels of sIL-2R in WGLF of patients with inactive disease were largely not significantly different from controls except for the inactive Crohn's

disease group (p=0.0064). This group gave even borderline significantly higher levels of sIL-2R in WGLF than patients with inactive UC (Table 12B:1E, p=0.042). This is explained by the fact that this inactive CD group had a preponderance of patients who were in the higher range of normal with regard to disease activity, and their WGLF total IgG was significantly higher than for inactive UC (p=0.0420) [see Table 12B:4C].

LEVELS OF sIL-2R IN WGLF ACCORDING TO REGIONAL INVOLVEMENT OF CROHN'S DISEASE

Patients were subdivided into subgroups as for serum sIL-2R. Patients with small bowel Crohn's disease had a range <10-350U/ml with a median of 68U/ml. The ileocolonic Crohn's disease group gave a range of <10-234U/ml with a median of 49U/ml. Patients with colonic Crohn's disease gave a range of <10-347U/ml with a median of 45U/ml, and the patients with Crohn's proctitis gave a range of 18-249U/ml with a median of 69U/ml. All these values were significantly higher than controls (p=0.0020, 0.0116, 0.0263 and 0.0288 respectively) [Table 12B:2A and Graph 12B:2].

The patients were further analysed according to the prevalence of high levels of sIL-2R in WGLF (>70U/ml). Out of 20 controls, 2 had high levels compared to 6 out of 15 patients with small bowel Crohn's disease, 5 out of

13 patients with ileocolonic Crohn's disease, 2 out of 10 patients with colonic Crohn's disease and 3 out of 6 patients with Crohn's proctitis (Table 12B:2B). The patients were further subgrouped into active and inactive disease groups, and similarly analysed. The statistical analyses are discussed below.

REGIONAL INVOLVEMENT, DISEASE ACTIVITY AND WGLF sIL-2R LEVELS IN CROHN'S DISEASE

All Crohn's disease subgroups with active disease had significantly higher levels of WGLF sIL-2R than controls. Patients with active small bowel Crohn's disease gave a range of 10-181U/ml with a median of 66U/ml ($p=0.0089$), active ileocolonic Crohn's disease gave a range of 10-167U/ml with a median of 87U/ml ($p=0.0358$), active colonic Crohn's disease gave a range of 10-347U/ml with a median of 48U/ml ($p=0.0348$) and active Crohn's proctitis gave a range of 46-249U/ml with a median of 79U/ml ($p=0.0223$) [Table 12B:2B and Table 12B:2C].

Only two inactive groups, inactive small bowel Crohn's disease and inactive ileocolonic Crohn's disease gave enough numbers for statistical evaluation. Patients with inactive small bowel Crohn's disease had significantly higher levels of sIL-2R in their WGLF than controls, with a range of 10-350U/ml and a median of 73U/ml ($p=0.0263$). The inactive ileocolonic Crohn's disease

group whose range was 10-234U/ml with a median of 46U/ml, did not differ significantly from controls (p=0.0638) [Table 12B:2B and Table 12B:2D).

LEVELS OF sIL-2R IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS ACCORDING TO REGIONAL INVOLVEMENT

Ulcerative colitis patients were further subdivided according to the distribution of disease as for serum sIL-2R above.

Only the active UC proctitis group gave significantly higher values than controls with a range of 44-149U/ml with a median of 107U/ml (p=0.0095). There was no significant difference between the other subgroups and controls (Tables 12B:3A-D and Graph 12B:3). The prevalence of high levels of sIL-2R in WGLF was analysed according to disease distribution of ulcerative colitis (Table 12B:3B). Out of 20 controls, 2 had high levels compared to 3 out 11 patients with pancolitis, 1 out of three with left sided ulcerative colitis and 3 out of 8 with ulcerative proctitis. The same analysis was done for patients grouped according to disease activity.

COMMENT

All regional subgroups in CD gave significantly higher levels than controls except for the inactive groups.

When ulcerative patients were subgrouped according to regional involvement only, the active proctitis group gave significantly higher levels of sIL-2R in WGLF than controls (Table 12B:3C). The inclusion of 4 of these patients as ulcerative proctitis is not fully conclusive and they are being followed up as they may turn up to have Crohn's disease or indeed high levels of sIL-2R in patients with ulcerative colitis could be a feature of ulcerative proctitis.

COMPARISON OF DISEASE ACTIVITY BETWEEN DISEASE GROUPS

The difference between the two disease groups with respect to controls, could be due to one group having patients with more active disease. As disease activity was based on WGLF IgG, comparisons of the levels of gut lavage IgG were made between the two groups. The IgG level in patients with ulcerative colitis was between 1-120ug/ml with a median of 27ug/ml, and the range for the Crohn's disease was between 1-150ug/ml. These levels were not statistically different (p=0.7480). However active UC patients median 53ug/ml and a range of 19-120ug/ml had significantly higher WGLF IgG than active Crohn's disease (median 26ug/ml with a range of 11-150ug/ml; p=0.0196). Inactive Crohn's disease gave a median of 5ug/ml with a range of 1-10ug/ml which was significantly higher than the IgG values for inactive ulcerative colitis (median of

2.5ug/ml with a range of 1-8ug/ml; (p=0.0420) [Tables 12B:4A-C).

COMMENT

The patients with ulcerative colitis were well matched with the patients with Crohn's disease as regards disease activity as there was no significant differences in their IgG levels. Patients with active UC had significantly higher levels of total IgG in their WGLF than patients active Crohn's disease. This is interesting in view of the fact that active Crohn's disease patients gave higher levels of statistical significance compared to controls than did patients with active ulcerative colitis compared with controls (Table 12B:1C; p=0.0005 and p=0.0136 respectively). It indicates that sIL-2R measurement looks at a different aspect of disease activity from WGLF total IgG (see final discussion chapter 14).

SECTION 12C

CORRELATIONS

THE RELATIONSHIP BETWEEN DISEASE ACTIVITY AND LEVELS OF sIL-2R IN SERUM AND WGLF

Levels of sIL-2R in WGLF were correlated with the levels of WGLF total IgG (a measure of disease activity) for each disease subgroup.

There was no significant correlation between disease activity, as measured by WGLF IgG, and WGLF sIL-2R for the controls as well as the disease groups (Table 12C:1). The result for patients with ulcerative colitis ($r=0.486$ and $p=0.016$) is accounted for mainly by negative values.

Levels of sIL-2R in serum were correlated with the levels of WGLF total IgG (a measure of disease activity) for each disease subgroup.

There was a strong correlation between disease activity and serum sIL-2R for all the patients with Crohn's disease ($r=0.540$, $p=0.0001$), also for patients with active Crohn's disease ($r=0.474$, $p=0.017$) [Table 12C:1]. Other subgroups showed no significant correlation between serum sIL-2R and WGLF total IgG.

THE RELATIONSHIP BETWEEN sIL-2R LEVELS IN WGLF, SERUM AND DISEASE ACTIVITY IN REGIONAL INVOLVEMENT OF DISEASE

Crohn's disease and ulcerative colitis patients were subgrouped according to regional involvement as outlined above (disease distribution for serum sIL-2R). Correlation coefficients were done between their levels of sIL-2R in WGLF and total WGLF IgG. There was no significant correlation between these measurements for any of the groups (Tables 12C:2A-C).

Correlation coefficients were also performed between sIL-2R levels in serum and WGLF total IgG. Serum sIL-2R correlated with lavage IgG for colonic Crohn's disease ($r=0.757$, $p=0.018$) and Crohn's proctitis ($r=0.852$, $p=0.031$) [Table 12C:2A].

None of the subgroups with UC showed any strong correlation between WGLF IgG and sIL-2R (RESULTS NOT SHOWN).

When the groups were divided further into active and inactive subgroups there was no significant correlation between WGLF IgG and sIL-2R in WGLF or serum (Table 12C:2B and Table 12C:2C). The lack of significant correlation for serum sIL-2R in active colonic and Crohn's proctitis groups could be because fewer numbers were studied. The lack of correlation between WGLF total IgG and WGLF sIL-2R in the active and inactive proctitis subgroups is in keeping with the findings in the general

Crohn's and ulcerative colitis study group were no significant correlation was found (Table 12C:1). Furthermore the level of significant correlation for the whole 6 Crohn's proctitis group is borderline at p=0.031.

THE RELATIONSHIP BETWEEN WGLF sIL-2R AND SERUM sIL-2R

Serum levels of sIL-2R and WGLF levels of SIL-2R were correlated for the individual groups.
There was no strong correlation between serum sIL-2R and WGLF sIL-2R for all the groups (Table 12C:3A).

COMMENT

There was a strong correlation between disease activity as measured by lavage IgG and serum sIL-2R for the whole Crohn's disease group, active Crohn's disease, colonic and Crohn's proctitis (Table 12C:1 and 2A). This probably reflects the fact that Crohn's disease patients tend to have more complications such as fistula formation, intestinal obstruction and intestinal perforations, concomitant with panmural bowel disease. The panmural involvement may increase the leakage of sIL-2R from the bowel lumen into systemic circulation. A combination of these complications and the panmural character of the disease involvement in patients with IBD, may lead to the corresponding increase in serum sIL-2R.

The lack of correlation between disease activity and WGLF sIL-2R for any of the disease groups may reflect the fact that the latter is a measure of a different phenomenon, such as its involvement in the upregulation of the immune response as discussed in chapter 4A (the Schlepper effect) unlike WGLF total IgG which is a result of leakage from plasma and not directly involved with immune activity. The upregulation of the immune response may be involved in the immunopathogenesis of IBD.

SECTION 12D

COMPARISONS OF LEVELS OF sIL-2R BETWEEN UPPER AND LOWER GASTRO-INTESTINAL INVOLVEMENT WITH IBD

To determine whether the levels of sIL-2R were related to upper or lower gastrointestinal bowel involvement, analysis of sIL-2R levels were done with patients divided into two groups, small bowel and large bowel involvement regardless of diagnosis. Small bowel involvement patients were invariably patients with Crohn's disease. The patients with ileocolonic disease were excluded.

COMPARISON OF LEVELS OF sIL-2R IN SERUM OF PATIENTS WITH SMALL BOWEL AND LARGE BOWEL IBD

Controls gave a range of 171-544U/ml with a median of 235U/ml. Patients with small bowel disease Crohn's disease gave a range of 63-1484U/ml with a median of 664U/ml, which was significantly higher than the levels for the controls ($p = 0.0150$). Patients with large bowel Crohn's disease gave a range of 50-2845U/ml with a median of 804U/ml, which was significantly higher than for the controls ($p=0.0051$) [Table 12D:1A].

The prevalence of high levels within disease groups is shown in table 12D:1B. Out of 11 controls, only one had high levels of sIL-2R in serum, compared to 9 out of 13

patients with small bowel disease or 16 out of 29 patients with large bowel disease. The large bowel disease group included patients with Crohn's colitis and ulcerative colitis. The statistical analyses of these are given above. These patient groups, small bowel and large bowel involvement, were further subgrouped according to disease activity and the prevalence of high levels determined (Table 12D:1B). The results of the statistical evaluation of these are discussed below.

SERUM sIL-2R LEVELS IN SMALL BOWEL AND LARGE BOWEL IBD ACCORDING TO DISEASE ACTIVITY

Patients with active small bowel disease gave a range of 63-1484U/ml with a median of 638U/ml, which was significantly higher than the levels for the controls ($p=0.0186$). The patients with large bowel disease gave a range of 50-2845U/ml with a median of 831U/ml, which was significantly higher than for the controls ($p=0.0014$) [Table 12D:1C]. There was no significant difference between the inactive disease groups and controls (Table 12D:1B and Table 12D:1D).

Comparisons in the levels of serum sIL-2R were made between large bowel and small bowel involvement. There was no significant difference in the serum levels of sIL-2R between the disease groups regardless of disease activity (Table 12D:1E).

LEVELS OF sIL-2R IN WGLF OF PATIENTS WITH SMALL BOWEL AND LARGE BOWEL DISEASE

The level of sIL-2R in the WGLF of patients with small bowel disease or large bowel disease were compared with levels in controls.

As before patients with small bowel disease gave a range of <10-350U/ml with a median of 68U/ml, which was significantly higher than the levels in controls ($p=0.0020$). Patients with large bowel disease gave a range of <10-347U/ml and a median of 42U/ml, which was significantly higher than for controls ($p=0.0172$) [Table 12D:2A].

The prevalence of high levels of sIL-2R in WGLF in small bowel disease and large bowel disease was evaluated. Out of 20 controls, 2 had high levels of sIL-2R in their WGLF compared to 6 out of 15 with small bowel disease or 10 out of 34 with large bowel disease (Table 12D:2B). Patients with large bowel disease included Crohn's colitis and ulcerative colitis. These patients were further subgrouped into active and inactive disease and their prevalence of high levels of sIL-2R in WGLF fluid analysed (Table 12D:1B). For example in patients with active disease 3 out 9 patients with small bowel involvement had high levels of sIL-2R in WGLF, as did 9 out of 22 patients with large bowel disease. The statistical evaluations of these patient groups compared

to controls are described below.

LEVELS OF sIL-2R IN WGLF OF IBD PATIENTS WITH SMALL BOWEL AND LARGE BOWEL IBD ACCORDING TO DISEASE ACTIVITY

Active disease groups had significantly higher levels of sIL-2R in their WGLF than controls. For small bowel disease the range was <10-181U/ml with a median of 66U/ml, and for large bowel the range was <10-347U/ml with a median of 55U/ml, both significantly higher than for controls ($p=0.0089$ and $p = 0.0046$, respectively) [Table 12D:2B and Table 12D:2C]. There were only 9 patients with small bowel disease involvement as compared to 22 patients with large bowel disease involvement; hence the greater significance with the large bowel group as compared to the small bowel group. However the medians and means were higher for the small bowel group (Table 12D:2C).

Patients with inactive small bowel disease gave a range of <10-350U/ml with a median of 73U/ml, which was significantly higher than the levels for controls ($p=0.0263$) while levels of sIL-2R in WGLF of patients with inactive large bowel disease gave a range of <10-73U/ml with a median of 34U/ml, which was not significantly higher than the levels for controls ($p=0.4363$) [Table 12D:2B and Table 12D:2D].

COMPARISON OF sIL-2R LEVELS IN WGLF BETWEEN SMALL BOWEL AND LARGE BOWEL INVOLVEMENT OF IBD

Comparisons in the levels of sIL-2R in WGLF were made between small bowel disease involvement and large bowel disease involvement (Table 12D:2E). There was no significant difference between the large bowel disease group taken as a whole compared to the levels in all the patients with small bowel disease involvement ($p=0.0701$). The levels in patients with active small bowel disease did not differ significantly from those with large bowel disease ($p=0.5423$). Patients with inactive small bowel disease gave a range of <10-350U/ml with a median of 73U/ml and the patients with inactive large bowel disease gave a range of <10-73U/ml with a median of 34U/ml. The levels in the small bowel group were higher than for the large bowel group with a borderline significance ($p=0.0492$) [Table 12D:2E].

COMMENT

These results show that sIL-2R levels were higher in the SB group on average than the large bowel group and the difference became significant when inactive small bowel patients are compared with inactive large bowel IBD patients ($p = 0.0492$) [Table 12D:2A-D]. The higher levels of sIL-2R in upper gastrointestinal involvement could be

because there is more lymphoid tissue in this region especially the ileum. Secondly as this is the area most exposed to exogenous antigens, immune activity would be expected to be higher in this region. In addition to this, by the time antigen such as polypeptides have reached the distal part of the intestines, a larger proportion of it will have been processed into less antigenic forms such as amino acids, as compared to the higher exposure to polypeptides in the upper gastro-intestinal tract. Increased immune activity would be reflected by an increase in the levels of sIL-2R, a receptor whose concentration increases when there is an immune response (see chapter 2A).

Further examination of the levels of sIL-2R in WGLF of patients with active and inactive small bowel disease yields some interesting paradox. Active small bowel disease gave a range of <10-181U/ml with a mean and median of 74U/ml and 66U/ml respectively (Table 12D:2C). The patients with inactive small bowel disease gave a range of <10-350U/ml with both the mean and median being 73U/ml (Table 12D:2D). This is interesting as it may suggest that high levels of sIL-2R are facilitatory in disease remission. If this were the case it would tend to render support to the suggestion by Smith and Cantrell (1985) and Rubin et al.(1986) that sIL-2R serves to down regulate the immune response. However it could equally suggest that the high levels are in keeping with the

result of an appropriately effective upregulated immune response which leads to the elimination of the offending antigen.

Levels of sIL-2R in patients with active large bowel disease gave a range of <10-347U/ml, a mean of 71U/ml with a median of 55U/ml (Table 12D:2C) which were higher than the inactive large bowel disease (range <10-73U/ml, mean 27U/ml with a median of 34) [Table 12D:2D]. This could indicate that high levels of sIL-2R are a feature of active disease. The lower levels in active small bowel disease than in inactive small bowel disease could be a result of increased degradation of sIL-2R in active small bowel disease compared to inactive small bowel disease. The source of these proteolytic enzymes apart from the lumen itself, which applies for both active and inactive small bowel disease, could be from the increased leakage of serum from systemic circulation (see chapter 7), and also the enzymes released from polymorphonuclear cells that leak into the bowel lumen in active disease (Saverymuttu et al., 1985b and 1985c). The effect of these enzymes would be greater for small bowel disease compared to large bowel disease due to the increased transit time for WGLF from the small bowel as compared from the large bowel. This question could be resolved by segmental sampling of the upper intestine perhaps as performed by Marteau et al. 1990, and comparing the levels of sIL-2R between active and inactive small bowel

disease groups. It is probable that patients with active disease would have higher levels of sIL-2R in their intestinal secretions than those with inactive disease.

As discussed above a number of suggestions have been made regarding the role of sIL-2R in the immune response and none has been proven. In view of this the results of treatment with elemental diet with respect to levels of sIL-2R may be quite informative. This point is discussed further with respect to the response to elemental diet below.

SECTION 12E

LEVELS OF sIL-2R in WGLF AND SERUM OF PATIENTS WITH GLUTEN RELATED ENTEROPATHY (GRE)

Patients with coeliac disease or dermatitis herpetiformis were grouped together as a gluten-related enteropathy group because both these groups do show enteral hypersensitivity to gluten.

Levels of sIL-2R were studied in the serum and WGLF of eight patients with gluten-related enteropathy. Seven of these patients had coeliac disease and were on a gluten free diet. The eighth patient had dermatitis herpetiformis and he was on a gluten free diet as well.

All the patients in the gluten-related group (GRE) were in disease remission.

SERUM LEVELS OF sIL-2R IN GRE PATIENTS COMPARED TO IBD PATIENTS AND CONTROLS

There were four serum specimens studied from this group. The range was 172-1396U/ml with a median of 289U/ml. This was not significantly different from either controls ($p=0.7441$) or patients with ulcerative colitis ($p=0.3350$) or Crohn's disease ($p=0.4160$). The median and range for the patients with gluten-related enteropathy (289U/ml and 172-1396U/ml respectively) were lower than that of

patients with ulcerative colitis (461U/ml and 60-1986U/ml respectively) or for Crohn's disease (611U/ml and 10-2844U/ml respectively). The median and range for the GRE group however were higher than for the controls whose median and range were 235U/ml and 171-544U/ml respectively (Table 12E:1A).

Comparisons in the serum levels of sIL-2R between the GRE group and patients with active ulcerative colitis or active Crohn's disease were made. These showed that there was no significant difference between the gluten-related enteropathy group and active ulcerative colitis or active Crohn's disease ($p=0.2175$ and $p=0.249$ respectively; Table 12E:1B).

WHOLE GUT LAVAGE FLUID sIL-2R IN GRE COMPARED TO CONTROLS AND IBD GROUPS

Whole gut lavage was obtained from all the eight GRE patients and assayed for sIL-2R as described in chapter 6A. The levels of sIL-2R in WGLF of the GRE group were compared with those of controls and IBD patients (Table 12E:2A).

The range of the levels of sIL-2R in WGLF of GRE patients was <10-169U/ml with a median of 59U/ml. Patients with ulcerative colitis gave a range of <10-149U/ml with a median of 38U/ml, this was not significantly different from the GRE group ($p = 0.5734$). The patients with

Crohn's disease gave a range of <10-350U/ml with a median of 56U/ml, this was not significantly different from the GRE group (p = 0.7950). Even the sIL-2R levels in WGLF of controls with a range of <10-97U/ml and a median of 10U/ml, were not significantly lower than for the GRE group (p= 0.1281) [Table 12E:2A].

Comparisons in the levels of sIL-2R in WGLF were made between the GRE group and patients with active ulcerative colitis or Crohn's disease. Patients with active ulcerative colitis gave a range of <10-149U/ml with a median of 66U/ml, and patients with active Crohn's disease gave a range of <10-347U/ml with a median of 59U/ml both these levels were not significantly different from the GRE group (p=0.1429 and p=0.7371 respectively) [Table 12E:2B].

COMMENT

These results do not reflect findings in acute untreated (active) gluten enteropathy. The levels of sIL-2R in WGLF and serum were intermediate between the levels in patients with active Crohn's disease or active ulcerative colitis and controls. The levels of sIL-2R did not differ significantly from either active ulcerative colitis, active Crohn's disease or controls, an intermediate position as observed before for patients with inactive Crohn's disease or inactive ulcerative colitis. As stated

above, all these GRE patients were in remission so these findings may not apply to the patients with untreated active gluten-related enteropathy. Furthermore no strong statistical conclusions could be made for the serum results of the GRE group as only 4 sera were assayed for sIL-2R.

SECTION 12F

COMPARISON OF LEVELS OF sIL-2R IN IBD PATIENTS CLASSIFIED AS SMALL BOWEL, COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS WITH CONTROLS

The groups of patients analysed above as large bowel disease included both Crohn's colitis and patients with ulcerative colitis. Therefore the findings with respect to the large bowel could not be attributed either to large bowel Crohn's disease or ulcerative colitis. In order to resolve this question the large bowel group was further subdivided according to whether they had Crohn's colitis or ulcerative colitis. The comparison in the levels of sIL-2R with small bowel Crohn's disease and controls was again performed.

COMPARISON OF LEVELS OF SERUM sIL-2R IN IBD PATIENTS CLASSIFIED AS SMALL BOWEL, COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS WITH CONTROLS

The serum levels of sIL-2R in controls gave a range of 171-544U/ml with a median of 235U/ml. Patients with small bowel disease gave a range of 63-1484U/ml with a median of 664U/ml. Crohn's colitis patients gave a range of 304-2845U/ml with a median of 1077U/ml, and patients with ulcerative colitis gave a range of 60-1986U/ml with a median of 461U/ml. All these values were significantly higher than for controls ($p=0.0150$; 0.0014 and 0.039 respectively) [Table 12F:1A]

The prevalence of high level of sIL-2R in serum was further analysed in these groups. Levels of serum sIL-2R above 510U/ml were considered high (see above). Out of 11 controls, 1 had high levels compared to 9 out of 13 patients with small bowel Crohn's disease, 7 out of 9 patients with colonic Crohn's disease and 9 out of 20 patients with ulcerative colitis. The same analysis was done for patients with active disease. Out of 8 patients with active small bowel disease, 6 had high levels of serum sIL-2R, out of 7 patients with active colonic Crohn's disease, 6 had high levels just as 7 out 12 patients with active ulcerative colitis. Statistical evaluation (see below) showed that all these values were significantly higher than for controls. Three out of five

patients with inactive small bowel disease had high values, one of the two with inactive colonic Crohn's disease had a high value, as had 2 of the 8 with inactive ulcerative colitis. Statistical evaluation of the levels in these inactive group compared to controls, where applicable, showed no significant difference (see below).

COMPARISON OF LEVELS OF sIL-2R IN ACTIVE IBD PATIENTS CLASSIFIED AS ACTIVE SMALL BOWEL, ACTIVE COLONIC CROHN'S DISEASE AND ACTIVE ULCERATIVE COLITIS WITH CONTROLS

Patients were further subdivided according to whether they had active disease or inactive disease on global clinical assessment and total lavage IgG levels (see chapter 7).

Patients with active small bowel Crohn's disease gave a range of 63-1484U/ml with a median of 638U/ml, active colonic Crohn's disease patients gave a range of 304-2845U/ml with a median of 1084U/ml, and active ulcerative colitis patients gave a range of 50-1986U/ml with a median of 591U/ml. All these values were significantly higher than for controls ($p=0.0186$, 0.0021, and 0.0151, respectively) [Table 12F:1C].

Patients with inactive small bowel Crohn's disease gave a range of 162-847U/ml with a median of 680U/ml and inactive ulcerative colitis gave a range of 209-1195U/ml with a median of 359U/ml. Both these values were not

significantly different from controls (p=0.1408 and 0.4090, respectively) [Table 12F:1D]. There were only two patients with inactive colonic Crohn's disease and the statistical analysis for these were not done.

COMPARISON OF SERUM LEVELS OF SIL-2R BETWEEN ULCERATIVE COLITIS, SMALL BOWEL CROHN'S DISEASE AND COLONIC CROHN'S DISEASE

The levels of serum sIL-2R were compared between patients with small bowel Crohn's disease, colonic Crohn's disease and ulcerative colitis. Even when these groups were subdivided according to disease activity there was no significant difference (Table 12F:1E).

COMMENT

Though there was no significant difference between small bowel Crohn's disease, colonic Crohn's disease and ulcerative colitis regardless of disease activity a pattern emerges. Patients with Crohn's disease have higher medians and mean levels of serum sIL-2R than ulcerative colitis. For small bowel Crohn's disease, the mean and median serum sIL-2R were 649U/ml and 664U/ml respectively and for colonic Crohn's disease the mean and median were 1150U/ml and 1077U/ml respectively (Table 12F:1A). Ulcerative colitis gave a mean of 648U/ml with a

median of 461U/ml lower than the two Crohn's disease groups. This suggests that raised sIL-2R are a feature of Crohn's disease as a whole compared to ulcerative colitis. I suggest that with more numbers levels of statistical significance could be reached. However serum sIL-2R may reflect only part of sIL-2R (see chapter 2A). This is discussed further after the next section.

COMPARISON OF LEVELS OF WGLF sIL-2R IN IBD PATIENTS CLASSIFIED AS SMALL BOWEL, COLONIC CROHN'S DISEASE AND ULCERATIVE COLITIS WITH CONTROLS

Whole gut lavage fluid from 20 controls, 15 patients with small bowel Crohn's disease, 10 patients with colonic Crohn's disease and 24 patients with ulcerative colitis was assayed for sIL-2R by ELISA (see chapter 6A).
The level of sIL-2R in the WGLF fluid of controls gave a range of <10-97U/ml with a median of 10U/ml. The patients with small bowel disease gave a range of <10-350U/ml with median of 68U/ml, significantly higher than controls ($p=0.0020$) and the patients with colonic Crohn's disease gave a range of <10-347U/ml with a median of 45U/ml, significantly higher than controls ($p=0.0263$). Patients with ulcerative colitis gave a range of <10-149U/ml with a median of 39U/ml, and this was not significantly higher than for controls ($p=0.1124$) [Table 12F:2A].

The prevalence of high levels of sIL-2R in WGLF of the groups studied were analysed. Levels of sIL-2R in WGLF above 70U/ml were considered high (see above). Two out of the 20 controls had high levels, 6 out of the 15 with small bowel Crohn's disease, and 2 out the 10 patients with colonic Crohn's disease (Table 12F:2B). The values of these patients when analysed statistically were significantly higher than for controls (see Table 12F:2A. Eight out 24 ulcerative colitis patients had high values but when analysed statistically these were not significantly different from controls.

A similar analysis was done for patients with active disease which showed that 3 out of 9 patients with active small bowel Crohn's disease had high levels, 2 out of 8 patients with active colonic Crohn's disease and 7 out of 14 patients with active ulcerative colitis (Table 12F:2B). On statistical analysis (see below) these levels of WGLF sIL-2R were significantly higher than for controls. Of the inactive disease groups only the inactive small bowel Crohn's disease group, with 3 patients with high levels out of 6 patients, showed significant differences from controls. Of the 10 patients with inactive ulcerative colitis only 1 had high levels and this was not significant as shown below.

COMPARISON OF LEVELS OF WGLF sIL-2R IN ACTIVE IBD PATIENTS CLASSIFIED AS ACTIVE SMALL BOWEL, ACTIVE COLONIC CROHN'S DISEASE AND ACTIVE ULCERATIVE COLITIS WITH CONTROLS

Patients were further subgrouped according to disease activity based on global clinical assessment and WGLF total IgG.

Patients with active small bowel disease gave a range of <10-181U/ml with a median of 66U/ml, active colonic Crohn's disease patients gave a range of <10-347U/ml with a median of 48U/ml and patients with ulcerative colitis gave a range of <10-149U/ml with a median of 68U/ml. All these levels of sIL-2R in lavage were significantly higher than for controls (p=0.0089, 0.0348 and 0.0136, respectively) [Table 12F:2C]

Only patients with inactive small bowel Crohn's disease with a range of <10-350U/ml and a median of 73U/ml gave significantly higher levels than for controls (p=0.0263). The patients with inactive ulcerative colitis gave a range of <10-73U/ml with a median of 24U/ml and this was not significantly different from controls (p=0.6284). There were only two patients with inactive colonic disease and these were not included in the calculations. (Table 12F:2D).

COMPARISON OF WGLF LEVELS OF SIL-2R BETWEEN ULCERATIVE COLITIS SMALL BOWEL CROHN'S DISEASE AND COLONIC CROHN'S DISEASE

The levels of sIL-2R in WGLF were compared between disease groups. There were no significant differences in the levels of sIL-2R in WGLF between small bowel Crohn's disease, colonic Crohn's disease or ulcerative colitis (Table 12F:2E).

COMMENT

Compared to the results in serum the results in WGLF, which may reflect directly intestinal immune activity, (see chapter 4A; Mahida et al., 1990) show a more clear cut pattern. There are significantly higher levels in patients with Crohn's disease compared to controls whether the disease involves the small bowel or colon. The levels in WGLF of patients with ulcerative colitis however are not significantly higher than for controls. The mean and median levels are highest in patients with small bowel Crohn's disease (87U/ml and 68U/ml respectively), followed by those with colonic Crohn's disease (72U/ml and 45U/ml respectively) and lastly for the patients with ulcerative colitis with a mean of 49U/ml and a median of 39U/ml (Tables 12F:2A and 12F:2B). The level of significance compared to controls is higher

for small bowel Crohn's compared to controls (p=0.0020) than for colonic Crohn's compared to controls (p=0263). This suggests that high levels of sIL-2R are more a feature of Crohn's disease than ulcerative colitis and that even within Crohn's disease higher levels are associated more with small bowel disease.

That Crohn's disease should show higher immune activity than ulcerative colitis would be in keeping with the postulates made by Lewkonia and McConnel (1976) that patients with Crohn's disease may have inherited more of the relevant genes than those with ulcerative colitis.

This is in keeping with the results of chapter 9, which showed that the Crohn's disease patients are the 'hyper-immune' group. The next section is a study of T cell activity, as measured by levels of sIL-2R, in relation to elemental diet.

SECTION 12G

ELEMENTAL DIET AND SOLUBLE INTERLEUKIN-2 RECEPTOR IN WGLF AND SERUM

STUDY POPULATION

Fourteen patients were prescribed elemental diet in the clinical management of their gastrointestinal disease. Of the fourteen patients, 12 had Crohn's disease, one ulcerative pancolitis and one coeliac disease (Table 12G).

The patients were prescribed an exclusive elemental diet for at least 7 days. Serum and whole gut lavage fluid were collected before the introduction of the diet and after at least 7 days of exclusive elemental diet feeds. The specimens were assayed for sIL-2R by ELISA as described in chapter 6A. At the commencement of the diet all the IBD patients had active disease on global clinical assessment. The disease distribution in the patients with Crohn's disease was as follows; ileal disease (4), colitis (3), ileocolonic (3), perianal and rectal (1) and one with microscopic Crohn's disease.

Drug treatment within three weeks of commencement of the diet was as follows, no drug treatment at all (2), 5-amino salicylic acid only (5), prednisolone only (1), 5-amino salicylate and prednisone (3) and prednisolone

and colifoam (1). The patient with ulcerative colitis had been on a combination of 5-amino-salicylate, oral prednisolone and prednisolone enema and the coeliac disease patient had been on a gluten free diet and no other medication.
All the nutritional requirements were provided for by elemental diet supplying an average of 2000kcal per day. No other food supplements were allowed.

RESULTS

The levels of sIL-2R in WGLF and serum for specimens taken before and while on elemental diet are shown with patient data (Table 12G). These are described below.
On global assessment, the clinical condition of 4 of the IBD patients improved (responders), 5 showed no change and four deteriorated. Their results are plotted in graphs 12G:1 (serum levels) and 12G:II (WGLF levels). A pattern, as described, below tends to form.

SERUM sIL-2R and ELEMENTAL DIET

The levels of sIL-2R in serum before the commencement of the diet were highest in the patients who did not respond to elemental diet. Though the initial levels of sIL-2R in the sera of those who responded were higher than for the controls studied (median 235, mean 314 see

Table 12F:1D), the levels in these responders were much lower than those of the group that deteriorated. The concentration of sIL-2R in the sera of all the non responders rose while the levels in responders were more or less constant. In patients who showed no change, initial sIL-2R levels in serum were in the same range as for the responders except for one patient (DS) who had lower levels. In two the levels of serum sIL-2R rose (MI) and (DS). For the other two (AM) and (EM) the levels remained unchanged (Graph 12G:I).

WHOLE GUT LAVAGE FLUID sIL-2R AND ELEMENTAL DIET

The results in this intestinal fluid were more striking. All the four patients who responded to elemental diet therapy started with very high levels of sIL-2R in WGLF (above 100U/ml, higher than the high levels described in earlier sections of this chapter) and the levels were lower in the second specimens taken after the patients had been on an exclusive elemental diet for at least seven days.

The non-responders and those whose clinical condition remained static, had initial levels that were low below 100 and these levels did not fall in the specimens collected after at least seven days on an exclusive elemental diet. The patient with coeliac disease had already been on a gluten-free diet and her initial lavage

sIL-2R level in WGLF was 49U/ml. The level of sIL-2R in the second WGLF specimen after 28 days of elemental diet was 42U/ml. Her clinical condition did not change. More patients with more acute disease need to be studied. This patient is not discussed any further.

COMMENT

The finding in the responders of low initial levels of sIL-2R in serum and high initial levels in whole gut lavage fluid could indicate that in these responders the main site of disease was the gastrointestinal tract. The high serum and low WGLF levels of sIL-2R in patients whose clinical condition deteriorated could indicate that the main site of disease activity may not be intestinal or that their exacerbation is due to some phenomenon other an inflammatory stimulus such as infection.
The corollary to this hypothesis, is that interleukin-2 receptor itself, during reduced antigenic stimuli, plays a part in the efficacy of elemental diet by a negative feedback leading to its own down regulation, a mechanism of action not very dissimilar from that of cyclosporin (Brynskov and Tvede, 1990).

GENERAL COMMENT

The aim of these experiments was to study the pattern of sIL-2R in WGLF and serum in IBD patients. The results from the two fluids were different in some respects. For example in serum, patients with ulcerative colitis or Crohn's disease gave significantly higher levels of sIL-2R than for controls ($p=0.039$ and 0.0045, respectively) [Table 12A:1A]. The results of sIL-2R in WGLF fluid are different, with Crohn's disease giving highly significantly higher levels compared to controls ($p=0.0003$) but not the patients with ulcerative colitis ($p=0.0533$) [Table 12B:1A].

These differences between the trends shown by the WGLF and serum sIL-2R results could reflect the different sources of the sIL-2R in the two fluids. Serum sIL-2R may be primarily of systemic origin (from the systemic lymphoid system), the source of sIL-2R in lavage fluid is not clear. Evidence from other workers (Mahida et al., 1990 and Choy et al. 1990) suggests that it is mainly of local intestinal origin. There is an increased expression of IL-2R by cells in the gastrointestinal tract of patients with active IBD (Mahida et al., 1988; Choy et al., 1990). When Mahida et al. (1990) measured the levels of sIL-2R in the mesenteric vessels of IBD patients for whom they had peripheral blood as well, they found that the levels were higher in the mesenteric vessels. They

proposed that there was a significant contribution by intestinal mononuclear cells to the circulating sIL-2R concentration.

In this study lavage IgG correlated with serum sIL-2R for the Crohn's disease group but there was no strong correlation between lavage IgG and lavage sIL-2R. Whole gut lavage IgG is probably mainly from serum (Chapter 7), but the source of lavage sIL-2R has not been determined. It may be secreted by cells in the lamina propria, mesenteric lymph nodes or it may be of systemic origin. The lack of correlation with lavage IgG makes a systemic origin less likely in addition to the evidence from other workers sited above.

If then the sIL-2R in WGLF is of local origin, it would be a more accurate mirror of local immune activity than the levels of serum sIL-2R which are subject to other systemic conditions such as infections and other autoimmune diseases (Keicho et al., 1990; Koukkou et al., 1990 and WÜthrich et al., 1990). The lack of a clear demarcation between active and inactive IBD in WGLF levels could be attributed to the persistence of the upregulated immune activity in the clinically quiescent IBD. This is clinically important as it brings into question the criteria for stopping active treatment in patients who are in remission clinically.

There was no significant difference in the levels of sIL-2R in WGLF between ulcerative colitis and controls

(p=0.0533; Table 12B:1A) and even in blood the differences were more significant with Crohn's disease (p=0.0045) than for ulcerative colitis (p=0.039) [Table 12A:1B]. This pattern is as found in the experiments performed to find the hyperimmune group, with higher anti-food antibodies (see Chapter 9) suggesting that patients with ulcerative colitis occupy a midway position between controls and Crohn's disease in the degree of immune upregulation.

Whether high levels of sIL-2R are a feature of large bowel or small bowel disease is partly answered by the comparisons in the levels of sIL-2R between large bowel (Crohn's colitis and ulcerative colitis) involvement, small bowel Crohn's disease and controls. In all these comparisons, regardless of disease activity, the magnitude of significant difference in sIL-2R in WGLF between the two groups and controls is highest with the small bowel group (Table 12F:1A-E and Table 12F:2A-E).

The results of sIL-2R levels before and after elemental diet are interesting in view the reported type of patients who respond to elemental diet (see Chapter 5) and in view of the pattern of sIL-2R levels established in these series of experiments.

In the first place only 4 patients out of the 13 IBD patients responded which represents less than 33% response rate, much lower than the 80-90% reported in large trials (O'Morain et al., 1984; Okada et al., 1990

and see chapter 5). However this is explained by the fact that elemental diet in this study was being used for clinical reasons only as a tertiary treatment, after other treatment (steroids, aminosalicylates) had failed to induce a remission. The study was not designed as a trial of the efficacy of elemental diet in IBD which has been proved by others workers (see chapter 5).

It must be emphasised that the levels of sIL-2R in serum were also raised in the patients who responded, most likely reflecting a gastro-intestinal contribution of sIL-2R to the systemic circulation.

The interpretation of this very high initial levels of sIL-2R in the lavage of responders could be that it is a marker of immunological exacerbation of IBD which would be amenable to immunological treatment such as antigen withdrawal. Malchow et al. (1990) in their study comparing polymeric diets with standard steroid therapy found that the steroids were initially superior. They however observed that there was a cohort of patients that tended to respond to diet which needed defining. If these results are confirmed, with further studies these patients with high lavage sIL-2R would represent one such cohort.

It is also interesting to note that patients with Crohn's disease had relatively higher levels of sIL-2R in WGLF than patients with ulcerative colitis and even among these the small bowel Crohn's disease patients had

relatively higher levels than those with large bowel Crohn's disease. This supports previous observations that elemental diet was only effective in Crohn's disease and that small bowel Crohn's disease was more responsive than large bowel Crohn's disease. However the large 10yr review by Teahon et al. (1991) showed that this was not always the case, some patients with colonic Crohn's disease also responded to elemental diet. If high levels of sIL-2R in WGLF are associated with disease response as shown in this study then my finding of high lavage sIL-2R in some patients with ulcerative colitis as well as Crohn's colitis probably explains these apparently contradictory findings.

Once these findings of high lavage sIL-2R levels are confirmed, investigations towards the basic mechanisms of action of elemental diet would become feasible, thereby rationalising further the management of IBD.

SUMMARY

There were increased levels of sIL-2R in the blood and WGLF of patients with active IBD.

Whereas there was a strong correlation between disease activity as measured by lavage IgG and serum sIL-2R in Crohn's disease, there was no such correlation for WGLF sIL-2R.

High levels of soluble IL-2R as measured in WGLF were

associated mainly with upper gastrointestinal disease. The patients with ulcerative proctitis who gave significantly higher levels than controls form an interesting group that is being monitored. These patients may turn out to have Crohn's disease or as stated, high WGLF sIL-2R in ulcerative proctitis may be a distinguishing feature for this group.

Patients with high levels of soluble interleukin-2 receptor in WGLF responded to elemental diet. The mechanism of action of elemental diet remains unknown as does the immunopathogenesis of inflammatory bowel disease. Elemental diets could act by antigen withdrawal or antigen modification. The antigen could be dietary, intestinal flora or their immunostimulatory products.

This preliminary work has characterised a cohort of responders to elemental diet which would form a possible base for further investigations.

APPENDIX FOR CHAPTER 12

TABLE 12:1A STUDY POPULATION

	CROHN'S DISEASE	ULCERATIVE COLITIS	CONTROLS
NUMBERS	45	24	20
SEX M:F	24:21	10:14	7:13
AGE			
MEDIAN	44	38	46
RANGE	14-83	22-79	21-85

DISEASE DISTRIBUTION

CROHN'S DISEASE		ULCERATIVE COLITIS	
Jejunal	4	Rectum only	8
Terminal ileum	11	Left sided	3
Ileocolonic	13	Pancolitis	11
Colonic	10	Pouchitis	1
Rectal	5	Microscopic	1
Perianal	4	14 active and 10 inactive	
Microscopic	1		

28 Active and 17 inactive

10 resections

TABLE 12:1A continued

CONTROLS	
Polyps	2
Constipation	5
Abdominal pain (unexplained)	8
Hospital workers	3
Diarrhoea (unexplained)	1
Duodenal ulcer	1

DRUG TREATMENT

CROHN'S DISEASE		ULCERATIVE COLITIS	
No drugs	12	No Drugs	7
Prednisolone only (Pred)	10	PRED, ASA and CFM	3
Sulphasalazine (SLZ)	13	ASA and CFM	2
5-amino salicylates only (ASA)	0	ASA only	6
SLZ and Pred	7	ASA and Pred	3
Pred, ASA and colifoam (CFM)	1	ASA,CFM and Azathioprine	1
SLZ, PRED and CFM	1	ASA,Pred and Cyclosporin	1
Pred and CFM	1	CFM only	1

TABLE 12A:1B LEVELS OF SERUM IL2R (U/ml) IN PATIENTS WITH CROHN'S DISEASE (CD), ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
UC	20	60-1986	648	461	0.039
CD	38	10-2844	611	611	0.0045

p = probability that the difference is a chance finding.

TABLE 12A:1C THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE SERA OF PATIENTS WITH ULCERATIVE COLITIS (UC), CROHN'S DISEASE (CD) PATIENTS AND CONTROLS. sIL-2R levels above 510U/ml

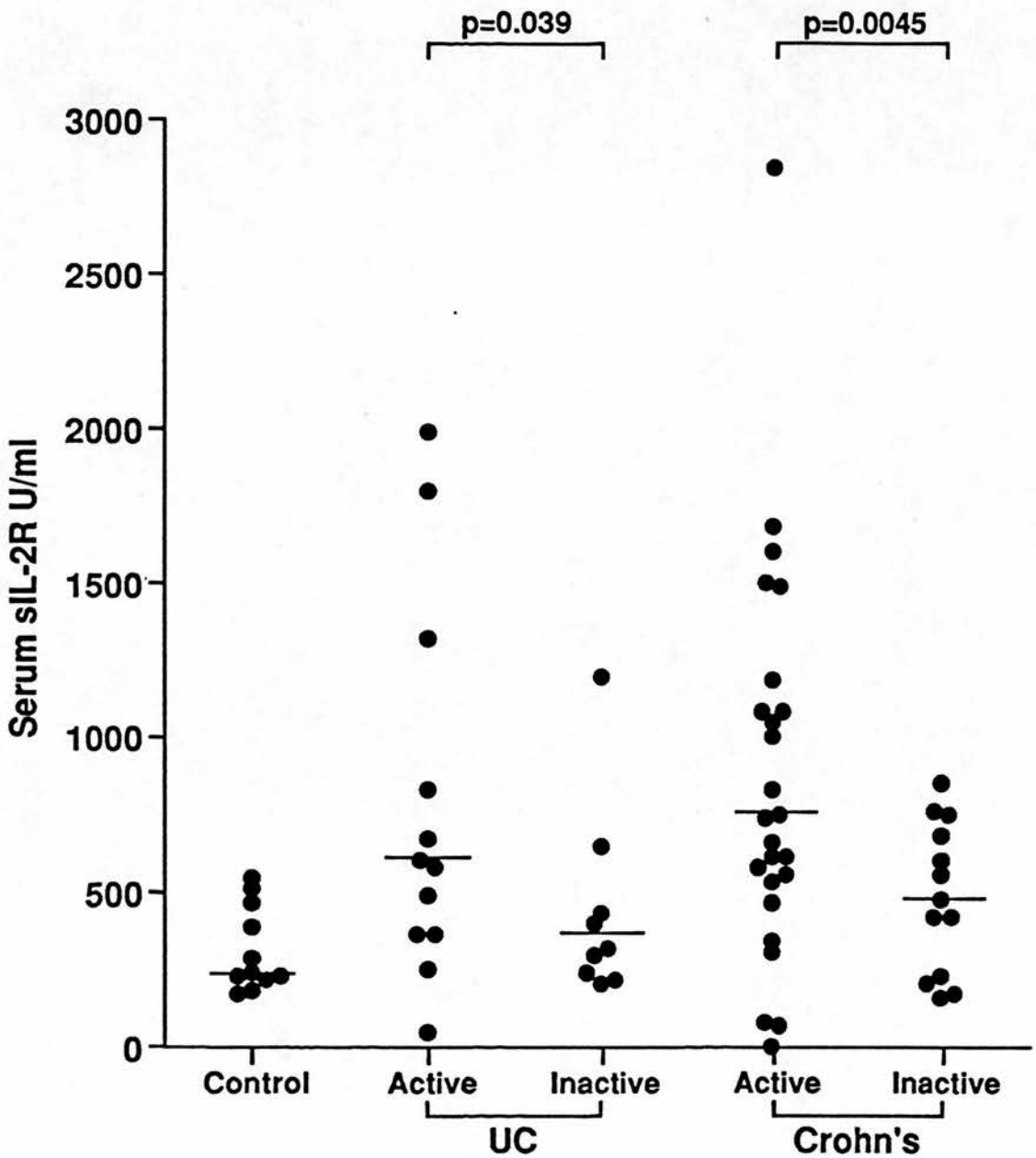
	ALL		ACTIVE		INACTIVE	
	N	>510	N	>510	N	>510
CONTROL	(11)	1		NA		NA
UC	(20)	9*	(12)	7*	(8)	2
CD	(38)	25**	(25)	19***	(13)	6

NA = Not applicable

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

Graph 12A:1

SERUM SOLUBLE INTERLEUKIN-2 RECEPTOR IN CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS



On the x axis are disease groups according to diagnosis and disease activity plotted against their levels of soluble interleukin-2 receptor in their sera. Both ulcerative colitis and Crohn's disease had significantly higher levels compared to controls and this was accounted for mainly by patients with active disease.

TABLE 12A:1D LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH ACTIVE CROHN'S DISEASE (CD), ACTIVE ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
UC	12	50-1986	775	591	0.0151
CD	25	10-2844	865	742	0.0016

p = probability that the difference is a chance finding.

TABLE 12A:1E LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH INACTIVE CROHN'S DISEASE (CD), INACTIVE ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
UC	8	209-1195	457	359	0.4090
CD	13	162-847	484	476	0.1320

p = probability that the difference is a chance finding.

TABLE 12A:1F COMPARISON OF LEVELS OF sIL-2R (U/ml) IN SERUM BETWEEN DISEASE GROUPS.

DISEASE GROUPS	p
UC vs CD	0.3904
aUC vs aCD	0.5702
inaUC vs inaCRO	0.6904
aUC vs inaUC	0.1535
aCD vs inaCRO	0.0526

p = probability that the difference is a chance finding.

TABLE 12A:2A LEVELS OF SERUM sIL-2R (U/ml) IN PATIENTS WITH CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT OF DISEASE.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	13	63-1484	649	664	0.0150
ILC	9	80-1181	545	580	0.1106
CN	9	304-2845	1150	1077	0.0014
PR	6	208-1597	704	516	0.0786

SB - Small bowel Crohn's disease CN - Colonic Crohn's disease

ILC - Ileocolonic Crohn's disease PR - Crohn's Proctitis

TABLE 12A:2B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE SERA OF CROHN'S DISEASE PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT.

	ALL		ACTIVE		INACTIVE	
	N	>510	N	>510	N	>510
CONTROL	(11)	1		NA		NA
SB	(13)	9*	(8)	6*	(5)	3
ILC	(9)	6	(5)	4	(4)	2
CN	(9)	7**	(7)	6**	(2)	1
PR	(6)	3	(4)	3*	(2)	0

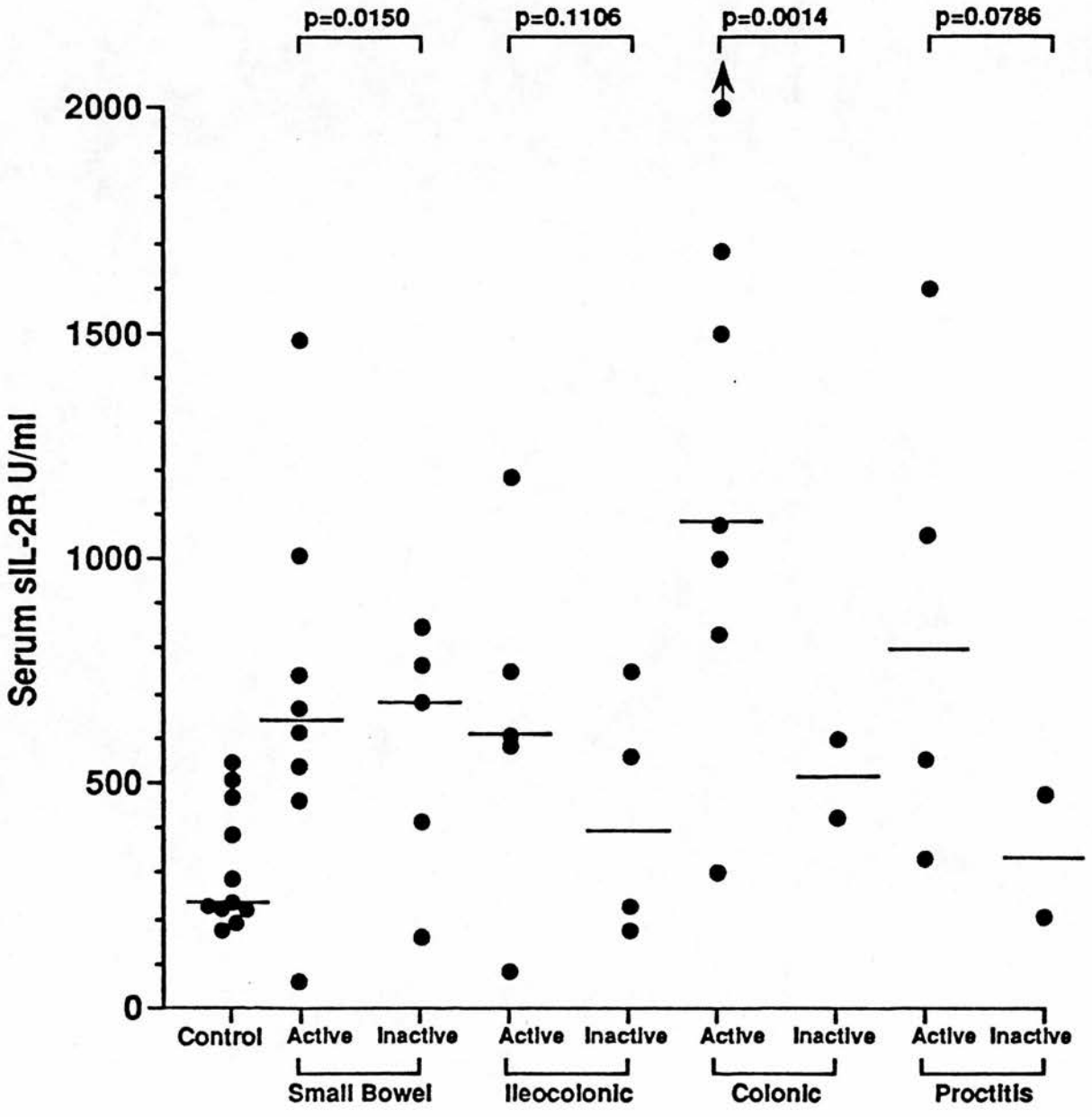
* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

N = Total numbers of patients studied in the group

p = probability that the difference is a chance finding.

Graph 12A:2

SERUM SIL2R IN CONTROLS AND CROHN'S DISEASE BY REGIONAL DISTRIBUTION OF DISEASE



Regional involvement of Crohn's disease (x axis) plotted against serum sIL-2R on the y axis. p values against controls.

TABLE 12A:2C LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH ACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	8	63-1484	696	638	0.0186
ILC	5	80-1181	640	609	0.0699
CN	7	304-2845	1333	1084	0.0021
PR	4	337-1597	885	802	0.0223

p = probability that the difference is a chance finding.

TABLE 12A:2D LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH INACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	5	162-847	575	680	0.1408
ILC	4	171-748	427	395	0.5569
CN	2	NA	512	NA	NA
PR	2	NA	342	NA	NA

NA = Not applicable

p = probability that the difference is a chance finding.

TABLE 12A:3A LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
PAN	10	209-1800	691	544	0.0265
LS	2	212-365	289	NA	NA
PR	6	236-1986	704	493	0.0444

PAN - Pancolitis LS - Left sided

PR - Proctitis

NA = Not applicable

p = probability that the difference is a chance finding.

TABLE 12A:3B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE SERUM OF ULCERATIVE COLITIS PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT. sIL-2R levels above 510U/ml.

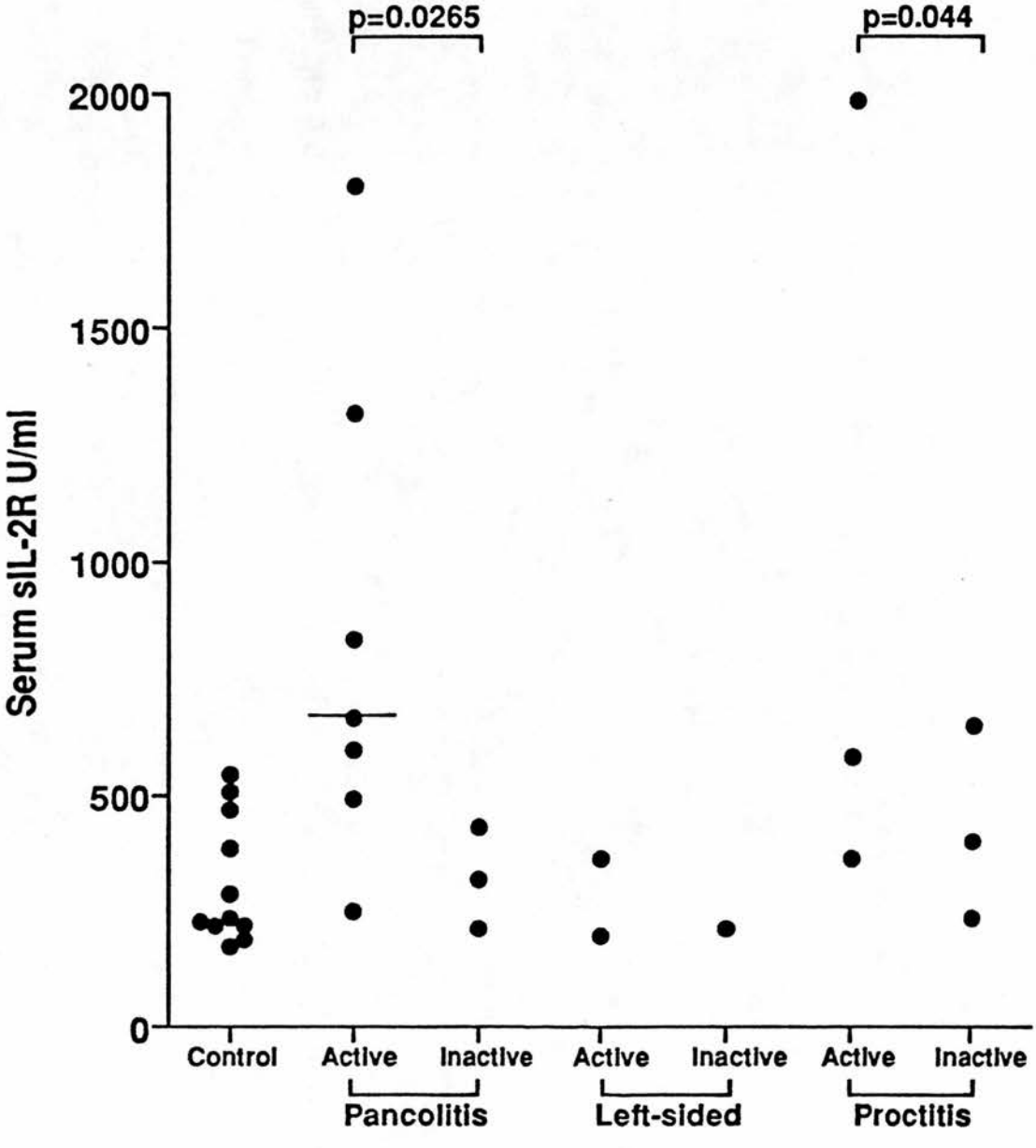
	ALL		ACTIVE		INACTIVE	
	N	>510	N	>510	N	>510
CONTROL	(11)	1		NA		NA
PAN	(10)	5*	(7)	5**	(3)	0
LS	(2)	0	(1)	0	(1)	0
PR	(6)	3*	(3)	2	(3)	1

N = Total numbers of patients studied in the group

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

Graph 12A:3

SERUM SIL2R IN ULCERATIVE COLITIS BY REGIONAL DISTRIBUTION OF DISEASE



Disease groups on the x axis according to regions affected by ulcerative colitis plotted against their levels of sIL-2R in serum on the y axis. Patients with pancolitis and proctitis had significantly higher levels than healthy controls. Patients with left sided disease were too few for firm statistical analysis.

TABLE 12A:3C LEVELS OF sIL-2R (U/ml) IN SERUM OF PATIENTS WITH ACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROLS	11	171-544	314	235	
PAN	7	249-1800	850	669	0.0050
PR	3	367-1986	978	583	0.0617

p = probability that the difference is a chance finding.

SECTION 12B

TABLE 12B:1A LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH CROHN'S DISEASE (CD), ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	<10	
UC	24	<10-149	49	38	0.0533
CD	45	<10-350	82	56	0.0003

p = probability that the difference is a chance finding.

TABLE 12B:1B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE WGLF OF ULCERATIVE COLITIS (UC) PATIENTS, CROHN'S DISEASE (CD) PATIENTS AND CONTROLS. sIL-2R levels above 70U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>70	N	>70	N	>70
CONTROL	(20)	2		NA		NA
UC	(24)	8	(14)	7*	(10)	1
CD	(45)	17***	(28)	10***	(17)	7**

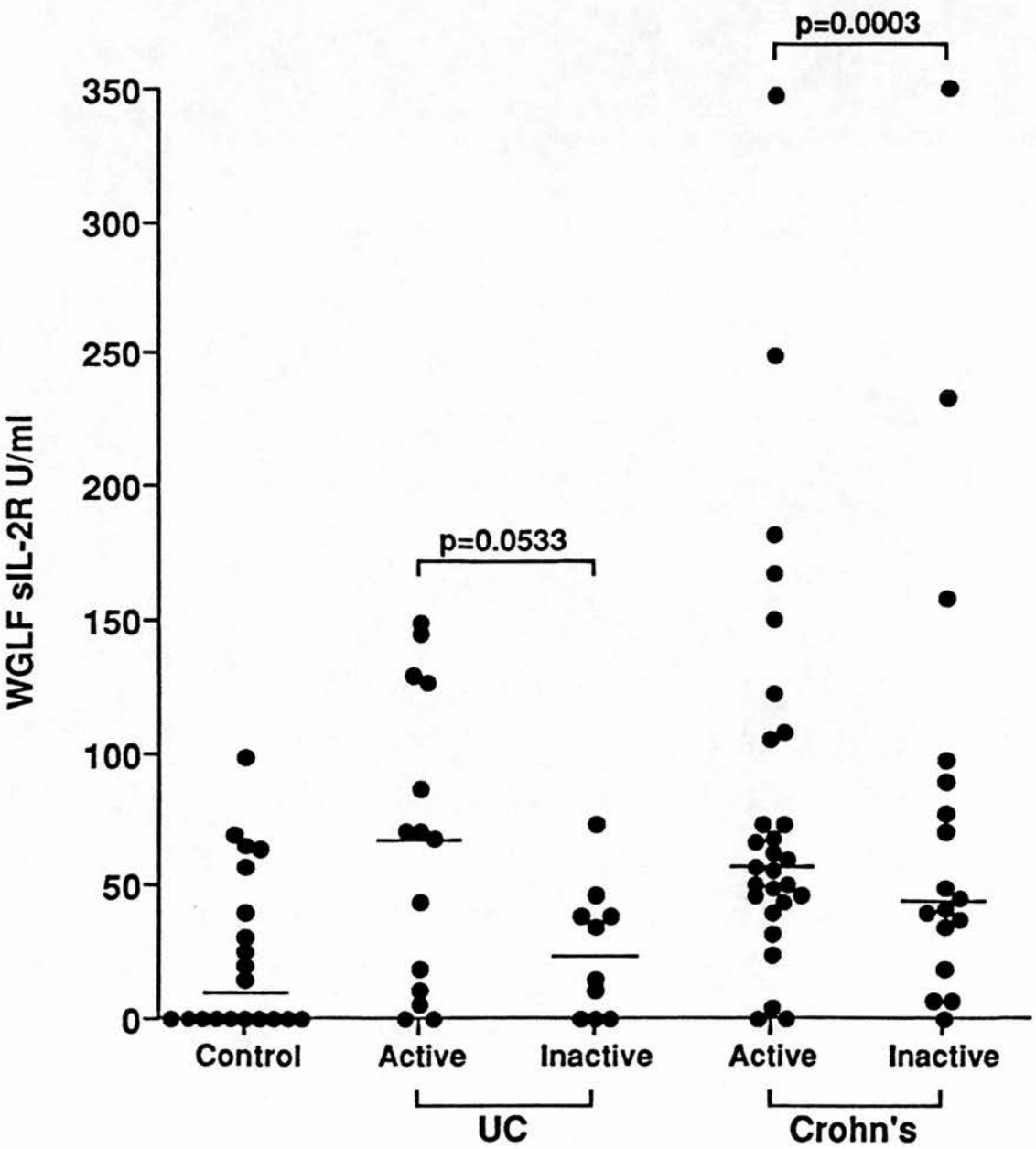
* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

N = Total numbers of patients studied in the group

Graph 12B:1

WHOLE GUT LAVAGE FLUID SOLUBLE INTERLEUKIN-2 RECEPTOR IN CONTROLS, CROHN'S DISEASE AND ULCERATIVE COLITIS



On the x axis are the subject groups plotted against their levels of sIL-2R in whole gut lavage fluid. All the patients with active disease (UC, CD) had significantly higher levels than controls as did the patients with inactive Crohn's disease.

TABLE 12B:1C LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE (CD), ACTIVE ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
UC	14	<10-149	66	68	0.0136
CD	28	<10-347	83	58	0.0005

p = Probability that the difference is a chance finding.

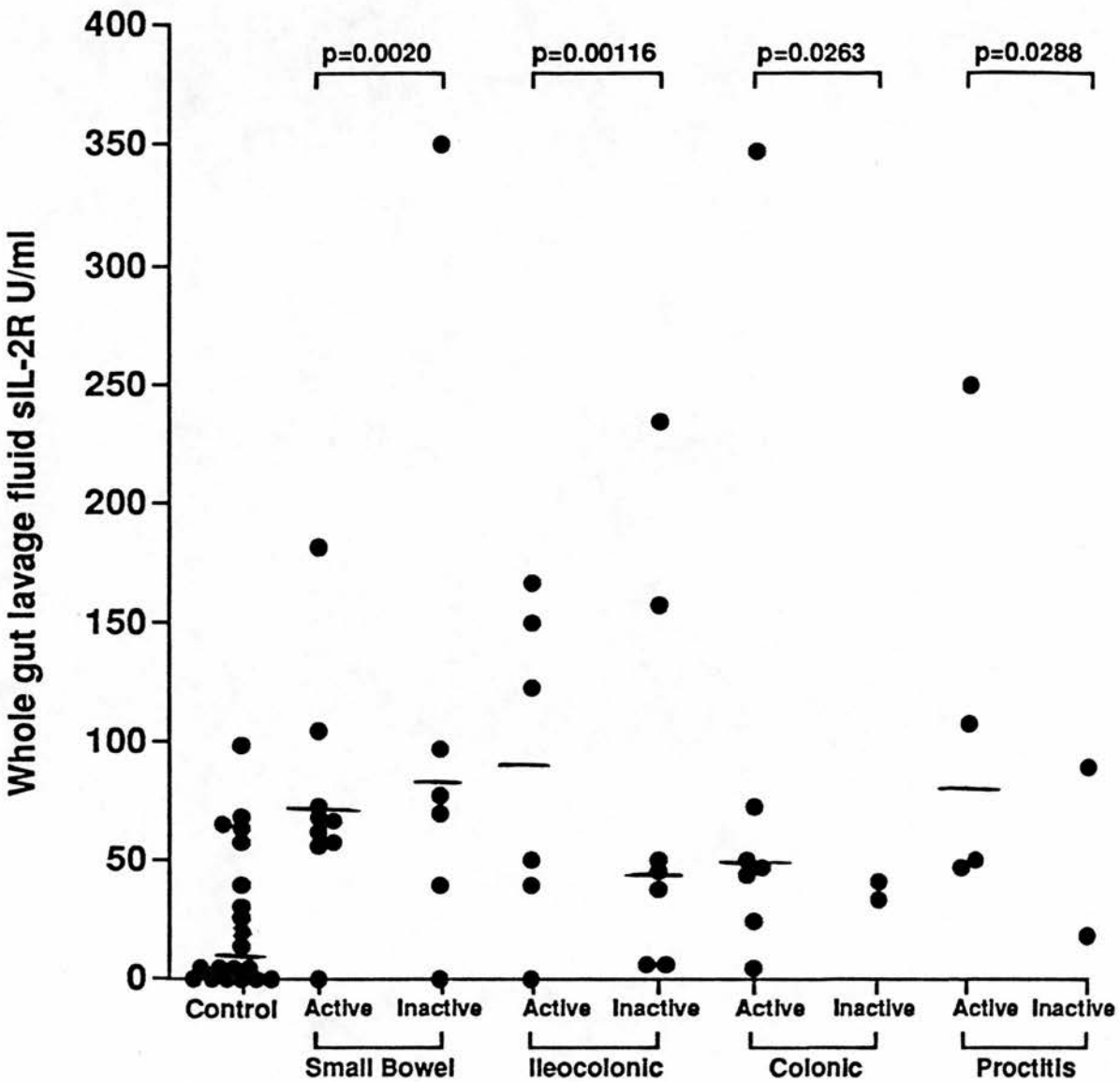
TABLE 12B:1D LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH INACTIVE CROHN'S DISEASE (CD), INACTIVE ULCERATIVE COLITIS (UC) AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
UC	10	<10-73	25	24	0.6284
CD	17	<10-350	80	45	0.0064

p = probability that the difference is a chance finding.

Graph 12B:2

WHOLE GUT LAVAGE FLUID SIL2R IN CONTROLS AND CROHN'S DISEASE BY REGIONAL DISTRIBUTION OF DISEASE



On the x axis are disease groups by regional involvement plotted agains their WGLF soluble interleukin-2 receptor on the y axis.
p values against controls

TABLE 12B:1E COMPARISON OF WGLF sIL-2R (U/ml) BETWEEN DISEASE GROUPS.

DISEASE GROUPS	p
UC vs CD	0.0659
aUC vs aCD	0.7387
InaUC vs inaCRO	0.0420*
aUC vs inaUC	0.0895
aCD vs inaCRO	0.4329

p = probability that the difference is a chance finding.

TABLE 12B:2A LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	15	<10-350	87	68	0.0020
ILC	13	<10-234	82	49	0.0116
CN	10	<10-347	72	45	0.0263
PR	6	18-249	93	69	0.0288

SB - Small bowel Crohn's disease PR - Crohn's Proctitis

ILC - Ileocolonic Crohn's disease CN - Colonic Crohn's disease

TABLE 12B:2B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE WGLF OF CROHN'S PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT. sIL-2R levels above 70U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>70	N	>70	N	>70
CONTROL	(20)	2		NA		NA
SB	(15)	6***	(9)	3**	(6)	3*
ILC	(13)	5*	(6)	3*	(7)	2
CN	(10)	2*	(8)	2*	(2)	0
PR	(6)	3*	(4)	2*	(2)	1

NA = Not applicable

N = Total numbers of patients studied in the group

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

TABLE 12B:2C LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	9	<10-181	74	66	0.0089
ILC	6	<10-167	88	86	0.0358
CN	8	<10-347	81	48	0.0348
PR	4	46-249	113	79	0.0223

p = probability that the difference is a chance finding.

TABLE 12B:2D LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH INACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	6	<10-350	73	73	0.0263
ILC	7	<10-234	46	46	0.0638
CN	2	NA	38	NA	NA
PR	2	NA	54	NA	NA

NA = not applicable

p = Probability that the difference is a chance finding

TABLE 12B:3A LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
PAN	11	<10-144	39	18	0.2079
LS	3	<10-70	28	14	0.7150
PR	8	<10-149	62	45	0.0984

PAN - Pancolitis PR - Proctitis LS - Left sided

p = Probability that the difference is a chance finding

TABLE 12B:3B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE WGLF OF ULCERATIVE COLITIS PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT. sIL2R levels above 70U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>70	N	>70	N	>70
CONTROL	(20)	2		NA		NA
PAN	(11)	3	(7)	2	(4)	1
LS	(3)	1	(2)	1	(1)	0
PR	(8)	3	(4)	3**	(4)	0

* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

N = Total numbers of patients studied in the group

TABLE 12B:3C LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
PAN	7	<10-144	45	18	0.2132
PR	4	44-149	102	107	0.0095

PAN - Pancolitis PR - Proctitis

p = Probability that the difference is a chance finding

TABLE 12B:3D LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH INACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

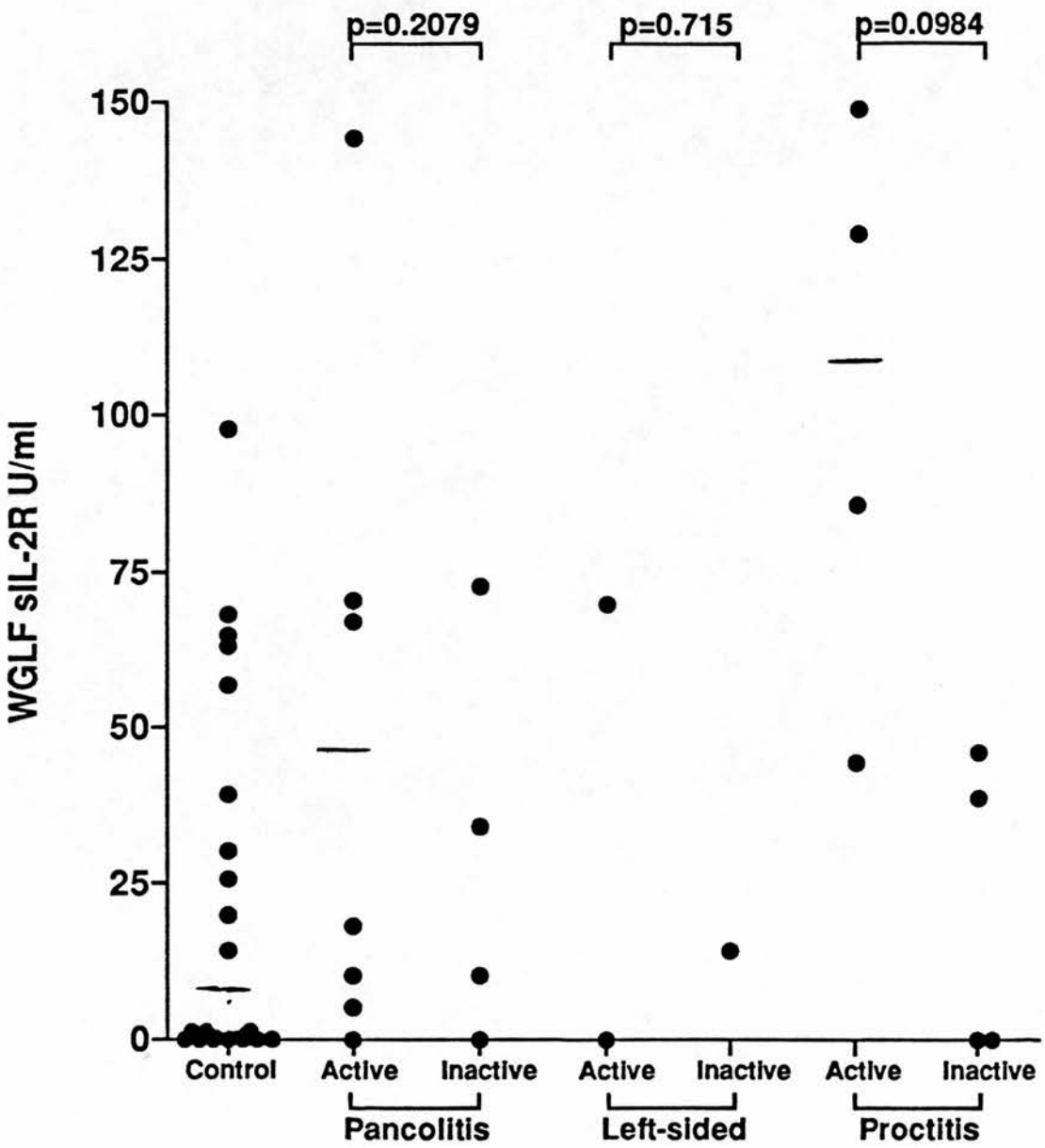
	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	29	10	
PAN	4	<10-73	21	22	0.5613
PR	4	<10-46		19	0.9691

PAN - Pancolitis PR - Proctitis

p = Probability that the difference is a chance finding

Graph 12B:3

WHOLE GUT LAVAGE SOLUBLE INTERLEUKIN-2 RECEPTORS IN CONTROLS AND ULCERATIVE COLITIS BY DISEASE DISTRIBUTION



Disease groups on the x axis according to regions affected by ulcerative colitis plotted against their levels of sIL-2R in whole gut lavage fluid on the y axis.

TABLE 12B:4A COMPARISON OF THE DISEASE ACTIVITY (WGLF total IgG ug/ml) BETWEEN ULCERATIVE COLITIS (UC) AND CROHN'S DISEASE (CD).

	N	RANGE	MEAN	MEDIAN	p
UC	24	1-120	38	27	
CD	45	1-150	27	15	0.7480

p = Probability that the difference is a chance finding

TABLE 12B:4B COMPARISON OF THE DISEASE ACTIVITY (WGLF total IgG ug/ml) BETWEEN ACTIVE ULCERATIVE COLITIS (UC) AND ACTIVE CROHN'S DISEASE (CD).

	N	RANGE	MEAN	MEDIAN	p
UC	14	19-120	63	53	
CD	28	11-150	40	26	0.0196

p = Probability that the difference is a chance finding

TABLE 12B:4C COMPARISON OF THE DISEASE ACTIVITY (WGLF total IgG ug/ml) BETWEEN INACTIVE ULCERATIVE COLITIS (UC) AND CROHN'S DISEASE (CD).

	N	RANGE	MEAN	MEDIAN	p
UC	10	1-8	3	2.5	
CRO	17	1-10	5	5	0.0420

p = Probability that the difference is a chance finding

CORRELATIONS

TABLE 12C:1 CORRELATIONS BETWEEN sIL-2R in WGLF , sIL-2R IN SERUM AND DISEASE ACTIVITY (WGLF total IgG)

GROUP	WGLF sIL-2R			Serum sIL-2R		
	N	r	p	N	r	p
CONTROL	20	-0.099	0.679	11	0.159	0.642
CRO	45	-0.041	0.789	38	0.540	0.0001*
UC	24	0.486	0.016+	20	0.104	0.664
aCRO	28	-0.055	0.782	25	0.474	0.017*
aUC	10	-0.316	0.271	8	-0.178	0.579
inaCRO	17	-0.494	0.044	13	-0.067	0.829
inaUC	10	-0.318	0.371	8	-0.444	0.270

+ Accounted for mainly by low results (negative results)

p = Probability that the correlation is a chance finding

TABLE 12C:2A CORRELATIONS BETWEEN WGLF sIL-2R, SERUM sIL-2R AND DISEASE ACTIVITY (WGLF total IgG) IN REGIONAL INVOLVEMENT OF CROHN'S DISEASE.

GROUP	WGLF sIL-2R			SERUM sIL-2R		
	N	r	p	N	r	p
SB	15	0.059	0.834	13	-0.288	0.340
ILCOL	13	-0.117	0.702	9	-0.203	0.601
COL	10	-0.035	0.923	9	0.757	0.0018*
PROCT	6	-0.038	0.943	6	0.852	0.031*

* significant correlation p > 0.05

TABLE 12C:2B CORRELATIONS BETWEEN WGLF sIL-2R, SERUM sIL-2R AND DISEASE ACTIVITY (WGLF total IgG) IN REGIONAL INVOLVEMENT OF PATIENTS WITH ACTIVE CROHN'S DISEASE.

GROUP	WGLF sIL-2R			SERUM sIL-2R		
	N	r	p	N	r	p
SB	9	0.620	0.075	8	-0.571	0.140
ILCOL	13	-0.254	0.627	9	-0.678	0.208
COL	6	-0.144	0.733	5	0.688	0.088
PR	4	-0.606	0.394	4	0.922	0.078

TABLE 12C:2C CORRELATIONS BETWEEN WGLF sIL-2R, SERUM AND DISEASE ACTIVITY (WGLF total IgG) IN REGIONAL INVOLVEMENT OF PATIENTS WITH INACTIVE CROHN'S DISEASE.

GROUP	WGLF sIL-2R			SERUM sIL-2R		
	N	r	p	N	r	p
SB	6	-0.350	0.496	5	0.126	0.840
ILCOL	7	-0.841	0.018	4	-0.010	0.990
COL	2	NA	NA	2	NA	NA
PR	2	NA	NA	2	NA	NA

NA = Not applicable

TABLE 12C:3A CORRELATIONS BETWEEN sIL-2R IN WGLF AND sIL-2R IN SERUM.

	N	r	p
CONTROL	9	0.285	0.396
CRO	38	0.139	0.404
UC	20	-0.214	0.366
GRE	8	0.799	0.201
aCRO	25	0.094	0.653
aUC	12	-0.416	0.179
inaCRO	13	0.297	0.325
inaUC	8	0.225	0.592

TABLE 12D:1A LEVELS OF sIL-2R (U/ml) IN SERUM OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) AND LARGE BOWEL (LB) INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	13	63-1484	649	664	0.0150
LB	29	50-2845	804	598	0.0051

p = Probability that the difference is a chance finding

TABLE 12D:1B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE SERA OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) AND LARGE BOWEL (LB) INVOLVEMENT. sIL-2R levels above 510U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>510	N	>510	N	>510
CONTROL	(11)	1		NA		NA
SB	(13)	9*	(8)	6*	(5)	3
LB	(29)	16**	19)	13***	(10)	3

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

SB - Small bowel Crohn's disease

LB - Large bowel involvement (ulcerative colitis and Crohn's colitis)

NA = Not applicable

TABLE 12D:1C LEVELS OF SERUM sIL-2R (U/ml) IN THE SERA OF ACTIVE IBD PATIENTS CLASSIFIED AS ACTIVE SMALL BOWEL (SB) AND ACTIVE LARGE BOWEL (LB) INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	8	63-1484	696	638	0.0186
LB	19	50-2845	981	831	0.0014

p = Probability that the difference is a chance finding

TABLE 12D:1D LEVELS OF sIL-2R (U/ml) IN THE SERA OF INACTIVE IBD PATIENTS CLASSIFIED AS INACTIVE SMALL BOWEL (SB) AND INACTIVE LARGE BOWEL (LB) INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	5	162-847	575	680	0.1408
LB	10	209-1195	468	413	0.2050

p = Probability that the difference is a chance finding

TABLE 12D:1E COMPARISON OF SERUM sIL-2R LEVELS BETWEEN SMALL BOWEL (SB) DISEASE AND LARGE BOWEL INVOLVEMENT (LB).

	ALL	ACT	INACT
SB vs LB	1.0000	0.8681	0.4260

P = Probability that the difference is a chance finding

TABLE 12D:2A LEVELS OF sIL-2R (U/ml) IN WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB), LARGE BOWEL (LB) INVOLVEMENT AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	15	<10-350	87	68	0.0020
LB	34	<10-347	56	42	0.0172

SB - Small bowel Crohn's disease

LB - Large bowel involvement includes Crohn's colitis and ulcerative colitis

P = Probability that the difference is not a chance finding

TABLE 12D:2B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) AND LARGE BOWEL (LB) INVOLVEMENT. sIL-2R levels above 70U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>70	N	>70	N	>70
CONTROL	(20)	2		NA		NA
SB	(15)	6***	(9)	3*	(6)	3
LB	(34)	10*	(22)	9**	(12	1

* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

N = Total numbers of patients studied in the group

TABLE 12D:2C LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ACTIVE IBD CLASSIFIED AS ACTIVE SMALL BOWEL (SB), ACTIVE LARGE BOWEL (LB) INVOLVEMENT AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	9	<10-181	74	66	0.0089
LB	22	<10-347	71	55	0.0046

p = Probability of the difference being a chance finding.

TABLE 12D:2D LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH INACTIVE IBD CLASSIFIED AS INACTIVE SMALL BOWEL (SB), INACTIVE LARGE BOWEL (LB) INVOLVEMENT AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	6	<10-350	73	73	0.0263
LB	12	<10-73	27	34	0.4363

p = Probability of the difference being a chance finding.

TABLE 12D:2E COMPARISON OF WGLF sIL-2R LEVELS BETWEEN INACTIVE SMALL BOWEL (SB) AND INACTIVE LARGE BOWEL (LB) INVOLVEMENT.

	ALL	ACT	INACT
SB vs LB	0.0701	0.5423	0.0492

p = Probability of the difference being a chance finding.

TABLE 12E:1A SERUM LEVELS OF sIL-2R (U/ml) IN GRE RELATED GROUP COMPARED WITH IBD AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	0.7441
UC	20	60-1986	648	461	0.3350
CD	38	10-2844	611	611	0.4160
GRE	4	172-1396	536	289	

p = Probability of the difference being a chance finding.

TABLE 12E:1B SERUM LEVELS OF sIL-2R (U/ml) IN GRE RELATED GROUP COMPARED WITH ACTIVE IBD PATIENTS.

	N	RANGE	MEAN	MEDIAN	p
aUC	12	50-1986	775	591	0.2175
aCRO	25	10-2844	865	742	0.2493
GRE	4	172-1396	536	289	

TABLE 12E:2A LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH GLUTEN RELATED ENTEROPATHY COMPARED WITH IBD AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	0.1281
UC	24	<10-149	49	38	0.5734
CD	45	<10-350	82	56	0.7950
GRE	8	<10-169	60	59	

p = Probability of the difference being a chance finding.

TABLE 12E:2B LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH GLUTEN-RELATED ENTEROPATHY COMPARED WITH PATIENTS WITH ACTIVE IBD.

	N	RANGE	MEAN	MEDIAN	p
aUC	14	<10-149	66	68	0.1429
aCRO	28	<10-347	83	58	0.7371
GRE	8	<10-169	60	59	

p = Probability of the difference being a chance finding.

COMPARISONS BETWEEN UPPER AND LOWER GASTROINTESTINAL INVOLVEMENT OF IBD.

TABLE 12F:1A LEVELS OF sIL-2R (U/ml) IN THE SERA OF PATIENTS WITH IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	13	63-1484	649	664	0.0150
CN	9	304-2845	1150	1077	0.0014
UC	20	60-1986	648	461	0.039

p = Probability of the difference being a chance finding.

TABLE 12F:1B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN THE SERA OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). sIL-2R levels above 510U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>510	N	>510	N	>510
CONTROL	(11)	1		NA		NA
SB	(13)	9*	(8)	6*	(5)	3
CN	(9)	7**	(7)	6**	(2)	1
UC	(20)	9*	(12)	7*	(8)	2

* p < 0.05 ** p < 0.01 *** p < 0.005

N = Total numbers of patients studied in the group

TABLE 12F:1C LEVELS OF sIL-2R (U/ml) IN THE SERA OF PATIENTS WITH ACTIVE IBD CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	8	63-1484	696	638	0.0186
CN	7	304-2845	1333	1084	0.0021
UC	12	50-1986	775	591	0.0151

p = Probability of the difference being a chance finding.

TABLE 12F:1D LEVELS OF sIL-2R (U/ml) IN THE SERA OF PATIENTS WITH INACTIVE IBD CLASSIFIED AS INACTIVE SMALL BOWEL (SB) CROHN'S, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	11	171-544	314	235	
SB	5	162-847	575	680	0.1408
CN	2	NA	512	NA	NA
UC	8	209-1195	457	359	0.4090

p = Probability of the difference being a chance finding.

TABLE 12F:1E COMPARISON OF sIL-2R LEVELS SERUM BETWEEN ULCERATIVE COLITIS (UC), SMALL BOWEL (SB) CROHN'S DISEASE AND COLONIC (CN) CROHN'S DISEASE.

	p SB vs UC	p SB vs CN	p UC vs CN
ALL	0.3866	0.1090	0.0562
ACT	0.8471	0.0562	0.1179
INACT	0.4208	NA	NA

NA = Not applicable

p = Probability of the difference being a chance finding.

TABLE 12F:2A COMPARISON OF LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH IBD CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	15	<10-350	87	68	0.0020
CN	10	<10-347	72	45	0.0263
UC	24	<10-149	49	39	0.1124

p = Probability of the difference being a chance finding.

TABLE 12F:2B THE FREQUENCY OF HIGH LEVELS OF sIL-2R IN WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). sIL-2R levels above 70U/ml.

	ALL		ACTIVE		INACTIVE	
	N	>70	N	>70	N	>70
CONTROL	(20)	2		NA		NA
SB	(15)	6***	(9)	3**	(6)	3*
CN	(10)	2*	(8)	2*	(2)	0
UC	(24)	8	(14)	7*	(10)	1

* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

N = Total numbers of patients studied in the group

TABLE 12F:2C LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH ACTIVE IBD PATIENTS CLASSIFIED AS ACTIVE SMALL BOWEL (SB) DISEASE, ACTIVE COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	9	<10-181	74	66	0.0089
CN	8	<10-347	81	48	0.0348
UC	14	<10-149	66	68	0.0136

p = Probability of the difference being a chance finding.

TABLE 12F:2D LEVELS OF sIL-2R (U/ml) IN WGLF OF PATIENTS WITH INACTIVE IBD PATIENTS CLASSIFIED AS INACTIVE SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<10-97	24	10	
SB	6	<10-350	73	73	0.0263
CN	2	NA	38	NA	NA
UC	10	<10-73	25	24	0.6284

NA = Not applicable

p = Probability of the difference being a chance finding.

TABLE 12F:2E COMPARISON OF LEVELS OF sIL-2R IN WGLF BETWEEN ULCERATIVE COLITIS (UC), SMALL BOWEL (SB) CROHN'S DISEASE AND COLONIC (CN) CROHN'S DISEASE.

	p SB vs UC	p SB vs CN	p UC vs CN
ALL	0.1124	0.1204	0.4844
ACT	0.9247	0.2482	0.8645
INACT	0.0509	NA	NA

NA = Not applicable

p = Probability of the difference being a chance finding.

INTERLEUKIN-2 RECEPTOR AND ELEMENTAL DIET PATIENT DATA

NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	ALB	SIL2R	LTIGG	LTNF	LIL2R	HB	CRP	ESR	WT	ALB	SIL2R	LTIGG	LTNF	LIL2R
DM	29	F	1	* STEROID FAILURE	NONE	21	14	2.3	37	50	36	464	64	<1	181	13	1.5	15	49	38	507	35	<1	46
BS	28	M	UC	* STEROID FAILURE	NONE	10	11	10	90	46	34	489	120	<1	266	11	1.5	50	51	34	765	120	<1	41
DS	30	F	1	RECRUDESCENCE OF DISEASE	HEMICOLECTOMY	31	10	3.6	25	42	30	63	64	35	67	11	3.7	32	44	31	607	61	1	92
DB	19	M	1	* RECURRENT ACTIVE DISEASE	ILEOCOLECTOMY	21	11	5.5	63	52	36	680	3	<1	350	10	1.5	37	52	34	720	1	<1	32
AM	38	F	2	OBSTRUCTIVE SYMPTOMS CROHN'S DISEASE	PROCTOCOLECTOMY	21	13	3.6	34	68	36	580	19	<1	39	15	1.5	17	68	42	552	12	20	36
EW	25	F	3	RECURRENT ACTIVE DISEASE	NONE	14	11	2.0	83	45	34	1139	133	42	55	9.5	3.9	80	45	34	1084	131	105	60
MI	50	F	2	ACTIVE ILEAL DISEASE	RT HEMICOLECTOMY	21	14	1.5	15	–	31	550	1	10	1	13	1.5	11	–	30	1503	1	119	4
JM	54	F	3	RECURRENT ACTIVE DISEASE	SIGMOID RESECTION	14	11	10	46	66	32	1181	15	51	49	10	1.5	42	64	33	1673	12	<1	121
CC	16	M	1PA	PERSISTENTLY ACTIVE CROHN'S DISEASE	SUBTOTAL COLECTOMY	14	10	7	25	25	28	1597	47	217	47	15	1.5	33	25.5	31	1991	19	<1	43
SJ	26	M	3	ABDOMINAL PAIN AND DIARRHOEA	NONE	21	14	1.5	33	82	40	461	2	217	34	14	1.5	13	83	40	534	5	190	8
EM	51	F	2	SB OBSTRUCTION RELUCTANT TO TAKE STEROIDS	TERMINAL ILEOCTOMY	14	13	11	79	57	40	430	29	<1	57	12	16	92	60	40	515	22	<1	83
FH	65	M	1	* MULTIPLE ILEOCOLONIC STRICTURES	T/IL+RCOL	21	14	9	22	63	39	–	12	–	122	14	1.5	11	64	39	–	4	–	19
GB	68	M	MICR	STEROID FAILURE	NONE	14	12	3.5	8	–	37	–	10	–	32	14	2.5	9	–	38	–	5	–	46
ES	34	F	coel	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	43	650	3	<1	62	12	1.5	10	41	44	700	2	44	59

← BEFORE ELEMENTAL DIET → ← ON ELEMENTAL DIET →

KEY

1 = ILEAL CROHN'S DISEASE

1PA = ILEAL +PERIANAL CROHN'S

2 = ILEOCOLONIC CROHN'S DISEASE

3 = COLONIC CROHN'S DISEASE

MICR = MICROSCOPIC CROHN'S DISEASE

UC = Ulcerative colitis

COEL = COELIAC DISEASE

DOD = DAYS ON DIET

SIL2R = SERUM INTERLEUKIN-2 RECEPTOR

LIL2R = LAVAGE INTERLEUKIN-2 RECEPTOR

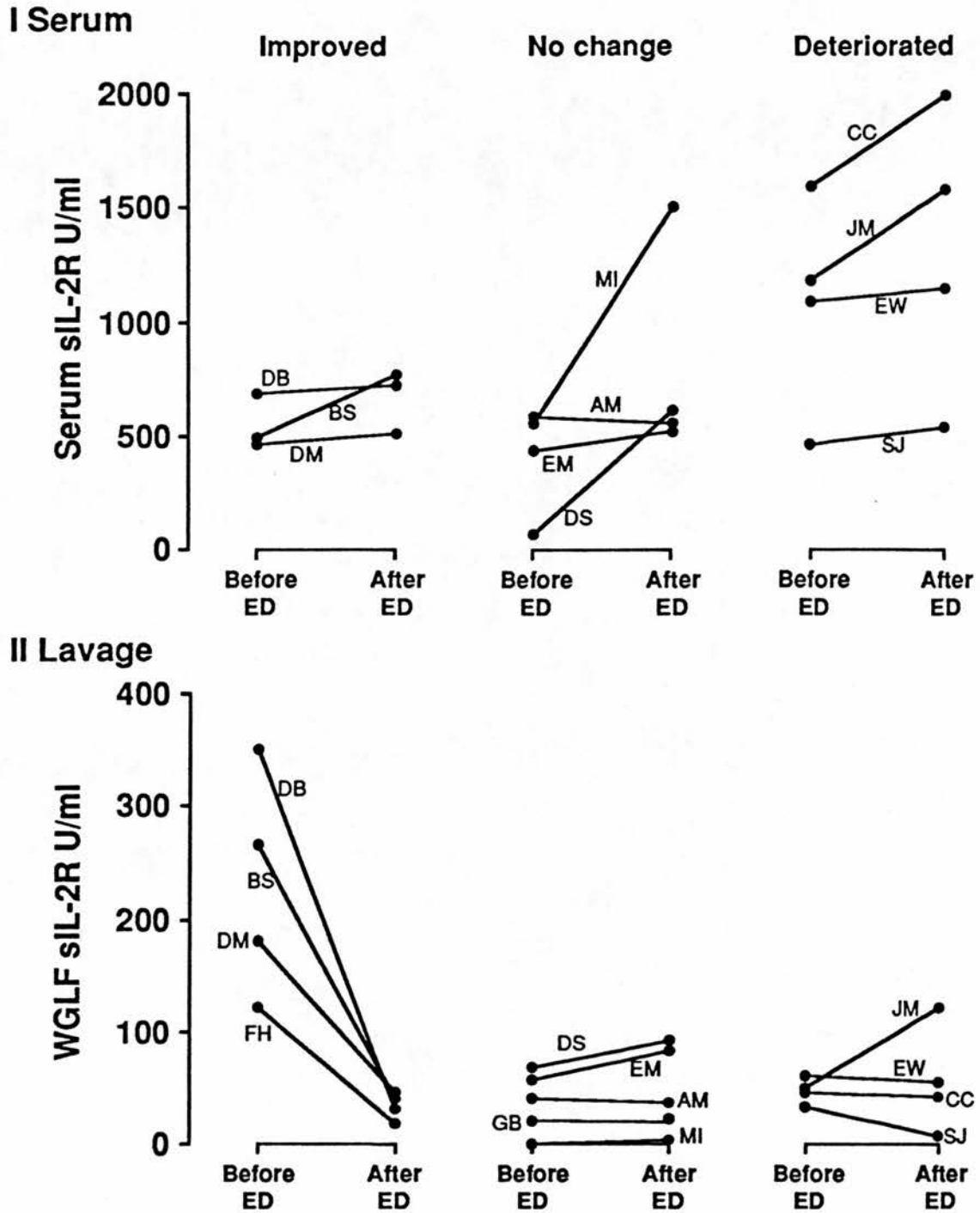
LTNF = LAVAGE TUMOUR NECROSIS FACTOR

LTIGG = LAVAGE TOTAL IgG

* = RESPONDERS

Graph 12G

SOLUBLE INTERLEUKIN-2 RECEPTOR AND ELEMENTAL DIET



Levels of soluble interleukin-2 receptor in serum (Top I) and whole gut lavage fluid (Bottom II) before and after at least seven days on elemental diet. On the y axes are the levels of IL-2R and on the x axes the time span. Patients are grouped according to global clinical response as improved, no change or deteriorated.

CHAPTER THIRTEEN:

TUMOUR NECROSIS FACTOR IN WHOLE GUT LAVAGE FLUID

INTRODUCTION

Tumour necrosis factor (TNF) is produced and secreted mainly by macrophages. These cells are abundant in intestinal mucosa. In IBD mucosa there are increased numbers of activated macrophages (Mahida et al., 1988) and activated T cells (Choy et al., 1990, see chapter 4B). The numbers of TNF secreting cells have been reported to be increased in the intestinal mucosa of IBD patients compared to controls (Macdonald et al., 1990). It is therefore not surprising that this cachexia causing substance (Tracey et al., 1988) has been implicated in the pathogenesis and complications of IBD (see chapter 4C). Tumour necrosis factor plays an important role in granuloma formation (Kindler et al., 1989), a feature of Crohn's disease. Attempts to study TNF activity in intestinal mucosa have been made difficult by the labile nature of TNF and the technical complexity of obtaining and preventing proteolysis in intestinal specimens. The little amount of work that has been done on TNF activity in patients with intestinal disease has relied on blood specimens which may not reflect intestinal mucosa TNF activity.

THE AIM OF THIS STUDY

The aims of this study were to investigate intestinal TNF

activity of individuals with healthy mucosa and diseased mucosa by measuring the levels of TNF in WGLF. This was performed in the following ways. The first part defined the pattern of TNF activity in relation to disease activity and regional distribution of disease in patients with IBD as compared to controls. The second part was a study of the relationship between the levels of whole gut lavage fluid TNF and other polypeptides assayed in the same lavage specimens such as sIL-2R and total IgG. Thirdly the relationship between intestinal TNF activity and response to elemental diet was studied by measuring the levels of TNF in WGLF of patients who had been prescribed elemental diet (as described below section).

SUBJECTS AND PROTOCOL.

The details of the subjects studied are shown in table 13:1A. The experiments were performed simultaneously with those for soluble interleukin-2 receptor in whole gut lavage fluid (WGLF) and serum, these results were reported in chapter 12.

Tumour necrosis factor in whole gut lavage fluid was assayed by ELISA as described in chapter 6A.

Comparisons of the levels of TNF were made between controls and IBD patients. Comparisons were also made after subdivision of groups according to disease activity

and regional distribution of the disease.

Correlation analyses were performed between WGLF IgG (a measure of disease activity), serum sIL-2R, WGLF sIL-2R and WGLF TNF to ascertain the relationship if any.

LEVELS OF TNF IN WGLF OF CONTROLS AND PATIENTS WITH IBD

Controls gave a range of <1-20pg/ml with a median of less than 1pg/ml (undetectable). Patients with ulcerative colitis gave a range of <1-184pg/ml with a median of 1, this was significantly higher than controls ($p=0.0420$). Similarly patients with Crohn's disease had a range of 1-217pg/ml with a median of 2pg/ml . This was significantly higher than for controls ($p=0.0064$) [Table 13A:1B and Graph 13:1 in the appendix to this chapter].

Analysis of the control results showed the value 10 was two standard deviations above the median and patients with values above 10 were considered to have high levels (Table 13A:1C). Out of 20 controls, 1 had high levels of TNF in their WGLF as compared to 8 out of 23 patients with ulcerative colitis and 16 out of 45 patients with Crohn's disease. Statistical analyses of their values showed that these were significantly higher than for controls (as above Table 13A:1B). For patients with active disease, 7 out of 13 patients with active ulcerative colitis had high values, and 9 out of 28 patients with Crohn's disease had high values, both these

results were significantly higher than for controls as shown below. Of the patients with inactive disease, 1 out of 10 patients with inactive ulcerative colitis had a high value and 7 out of 17 with inactive Crohn's disease had high values. Both these results when statistically analysed were not significantly different from controls.

LEVELS OF TNF IN WGLF OF PATIENTS WITH ACTIVE AND INACTIVE IBD

Disease activity was based on clinical assessment as well as WGLF total IgG (see chapter 7). Both active CD with a range of 1-184pg/ml and a median of 3pg/ml and active ulcerative colitis with a range of 1-217pg/ml and a median of 36pg/ml had significantly higher TNF levels than controls ($p=0.0072$ and $p=0.0034$ respectively) [Table 13A:1C and Table 13A:1D]. There was no significant difference between normal controls and inactive Crohn's disease ($p=0.0549$) or inactive ulcerative colitis ($p=0.1524$) [Table 13A:1C an Table 13A:1E].

Comparisons between disease groups showed that patients with active UC had higher levels than patients with inactive UC ($p=0.02$). There was no significant difference between UC and CD ($p=0.8458$), active UC and active CD ($p=0.2125$), inactive UC and inactive CD ($p=0.1524$) or between active CD and inactive CD ($p=0.8791$) [Table 13A:1F].

COMMENT

Both patients with ulcerative colitis and Crohn's disease gave higher levels of TNF in WGLF than controls. This is in keeping with the work of Macdonald et al. (1990) who showed by ELISPOT assay that there were higher number of TNF secreting cells in IBD mucosa than controls. Alstead et al. (1991) reported similar findings although they assayed for TNF in serum.

In this work patients were subgrouped into active and inactive disease groups. This showed that the significant differences for the whole groups (UC or CD) were accounted for by the higher values of the patients with active disease (active ulcerative colitis and active Crohn's disease). There was no significant difference between inactive disease groups and controls. This shows that the levels of TNF are higher with active disease.

LEVELS OF TNF IN WGLF BY REGIONAL INVOLVEMENT OF CROHN'S DISEASE

Crohn's disease patients were further subdivided according to the distribution of disease into four groups; small bowel (mouth to ileum with no colonic involvement), ileocolonic, colonic (no small bowel involvement) and Crohn's proctitis (PR).

Small bowel (SB) Crohn's disease had significantly higher

TNF levels than controls, range 1-63pg/ml with a median of 1pg/ml (p=0.0196). The colonic disease (CN) group gave a range of 1-217pg/ml with a median of 14pg/ml, significantly higher than for controls (p=0.0073). Patients with Crohn's proctitis gave a range of 1-217pg/ml with a median of 26pg/ml significantly higher than controls (p=0.0263). Patients with ileocolonic disease gave a range of 1-104pg/ml and a median of 1pg/ml (p=0.2240) and this was not significantly higher than controls (Table 13A:2A).

As for the analysis of disease groups above, the prevalence of high levels of TNF (above 10) was analysed in these regional subgroups of Crohn's disease. Of the 15 patients with small bowel Crohn's disease, 4 had high levels and of the 10 with colonic Crohn's disease, 5 had high levels. Four of the 6 with Crohn's proctitis had high levels, and 3 of the 13 with ileocolonic Crohn's disease had high levels. The values of TNF in these patients when compared to controls of whom 1 out the 20 had a high level, were significantly higher except for the subgroup with ileocolonic Crohn's disease as shown above. Analysis of the prevalence of high levels according to disease activity showed that, 1 out 9 patients with active small bowel Crohn's disease had high levels, 3 out of 8 patients with active colonic Crohn's disease had high levels and 1 out of the 6 patients with active ileocolonic Crohn's disease had a high level. On

statistical analysis (see below) all these values were significantly higher than controls except for the patients with ileocolonic disease. This is because the active ileocolonic had on average lower values than the active small bowel group, even though 1 out 6 of their group had a high value compared to 1 out 9 of the patients with active small bowel disease (see below). The prevalence of high levels in patients with inactive disease showed that 3 out of 6 patients with inactive small bowel disease had high levels and 2 out of 7 patients with inactive ileocolonic Crohn's disease had high levels. On statistical analysis these results were not significantly higher than the levels for control. This appears contradictory in view of the higher proportion of high positive compared to patients with active disease with the same disease distribution. However an examination of the figures shows that the median for both inactive small bowel and ileocolonic were very low. Both the two patients with inactive colonic disease had high levels of TNF in WGLF and none of two with inactive Crohn's proctitis had high levels (Table 13A:2B)

LEVELS OF TNF IN WGLF BY REGIONAL INVOLVEMENT OF CROHN'S DISEASE AND DISEASE ACTIVITY.

Patients with Crohn's disease were further subdivided according to regional involvement and disease activity. Active small bowel disease gave a range of 1-41pg/ml with a median of 1pg/ml significantly higher than controls (p=0.0359). Active colonic gave a range of 1-118pg/ml with a median of 5pg/ml significantly higher than controls (p=0.0371). Active proctitis gave a range of 20-217pg/ml with a median of 32pg/ml significantly higher than controls (p=0.0022). The active ileocolonic group gave a range of 1-51pg/ml with a median of 1.5pg/ml not significantly different from controls (p=0.4652).

There were only two patients with inactive colonic Crohn's disease and two with inactive proctitis, so these were not evaluated further. Patients with inactive small bowel Crohn's disease gave a range of 1-63pg/ml with a median of <1pg/ml, this was not significantly different from controls (p=0.1281). Patients with inactive ileocolonic Crohn's disease gave a range of 1-104 with a median of <1 and this was not significantly different from controls (p=0.2567) [Table 13A:2D].

COMMENT

All subgroups of Crohn's disease had significantly higher levels of TNF in WGLF than controls except for the ileocolonic group. In this group, as pointed out in chapter 12, seven of the patients had had resections and probably the actual area of inflammation was not as widespread as the term ileocolonic implies.
Again raised TNF was a feature of active disease as the inactive groups, where numbers allowed for comparison, had levels not significantly different from controls. Though there were only 4 patients with active proctitis, they had the highest levels a mean of 76 and a median of 32 ($p=0.0022$). This could be a specific feature of Crohn's proctitis or it could be due to the reduced transit time which gives less time for proteolytic degradation. This point is discussed further with respect to ulcerative proctitis below.

LEVELS OF TNF IN WGLF BY REGIONAL INVOLVEMENT OF ULCERATIVE COLITIS.

Ulcerative colitis patients were further subdivided according to the distribution of disease into three groups; pancolitis (involving the whole colon), left sided (LS) and ulcerative proctitis (PR).
Patients with pancolitis had significantly higher levels

of TNF in WGLF than controls, range of 1-184pg/ml with a median of 4pg/ml (p=0.0235). There were only three patients with inactive left-sided disease and their WGLF TNf levels were 1pg/ml, 3pg/ml and 73pg/ml. The patients with proctitis gave a range of 1-100pg/ml with a median of 1pg/ml not significantly different from controls (p=0.4924) [Table 13A:3A].

The patients with ulcerative colitis were further analysed for the prevalence of high levels of TNF (above 10pg/ml) in WGLF according to regional involvement of disease and disease activity. Four out of the 9 patients with ulcerative pancolitis had high levels of TNF in WGLF, and on statistical analysis of their levels they were significantly higher than for controls as above. Three of the 6 patients with active ulcerative pancolitis had high levels and analysis of their results showed that these were significantly higher than for controls (see below). One out of the three patients with pancolitis had a high level and this was one of the 2 patients with active left sided ulcerative colitis. Two of the 8 patients with ulcerative proctitis had high levels of TNF in their WGLF and these were the 2 of the four with active disease. Statistical analyses of these were applicable are discussed below.

LEVELS OF TNF IN WGLF OF ULCERATIVE COLITIS PATIENTS BY REGIONAL INVOLVEMENT AND DISEASE ACTIVITY.

Ulcerative colitis patients were further subgrouped according to disease activity based on global assessment and WGLF total IgG.

Active pancolitis patients had significantly higher TNF than controls, range 1-183pg/ml with a median of 46pg/ml ($p=0.0056$). There were only 2 patients with active left sided ulcerative colitis, this number was too small for statistical evaluation and they are not considered any further. The four active ulcerative proctitis patients gave a range of 1-100pg/ml, with a median of 21pg/ml. This was not significantly different from controls ($p=0.1752$).

Inactive pancolitis gave a range of 1-14pg/ml with a median of <1pg/ml, not significantly different from controls ($p=0.6701$). Patients with inactive ulcerative proctitis had barely detectable TNF level of 1pg/ml, not significantly different from control. There was only one patient with inactive left sided disease with a TNF level of 3pg/ml (Table 13A:3C and Table 13A:3D).

COMMENTS

The nine patients with pancolitis had significantly higher ($p=0.0235$; Table 13A:3A) levels of TNF than

controls and the level of significance increased despite the smaller numbers (6) with active pancolitis ($p= 0.0056$). The numbers in ulcerative colitis regional involvement subgroups were too small for statistical evaluations, the median levels of their TNF in WGLF was <1, barely detectable.

Of note here, is the result from the 4 patients with active ulcerative proctitis. Unlike the patients with active Crohn's proctitis, the levels of TNF in the WGLF of patients with active ulcerative colitis were not significantly higher than controls. Therefore the highly significant levels with Crohn's proctitis may not be explained on the basis of reduced transit time, thus giving shorter period for proteolytic degradation.

SECTION 13B

LEVELS OF TNF IN WGLF OF CONTROLS, SMALL BOWEL (SB) AND LARGE BOWEL (LB) IBD

Patients with inflammatory bowel disease were divided according to whether they had small bowel or large bowel disease. The small bowel disease group were invariably Crohn's disease patients and Crohn's colitis and ulcerative colitis comprised the large bowel group. The patients with ileocolonic Crohn's disease were not included.

Patients with small bowel IBD gave a range of <1-63pg/ml with a median of 1pg/ml. This was significantly higher than controls ($p=0.0196$). Patients with large bowel IBD gave a range of <1-217pg/ml with a median of 2pg/ml and this was significantly higher than controls ($p=0.0078$) (Table 13B:1A)

The prevalence of high levels of TNF in WGLF (above 10) was analysed as before. Four of the patients 15 with small bowel disease had high levels, and 13 of the patients with large bowel IBD had high levels. Both groups were significantly higher than controls as shown above. A similar analysis was done with patients further subgrouped according to whether they had active or inactive disease. One out of the 9 patients with active small bowel disease had a high level, and 10 the 21

patients with large bowel disease had high levels. Both these results were significantly higher than for controls (see below). Although only one of the 9 with active small bowel disease had a high level of TNF in WGLF, the other 8 levels of TNF close to 10, hence the significant difference from controls (Table 13B:1B and Table 13B:1C).

LEVELS OF TNF IN WGLF OF CONTROLS, SMALL BOWEL (SB) AND LARGE BOWEL (LB) IBD ACCORDING TO DISEASE ACTIVITY

Patients were further subgrouped according to disease activity based on global clinical assessment and WGLF total IgG.

Patients with active small bowel IBD gave a range of <1-41pg/ml with a median of 1pg/ml, significantly higher than controls (p=0.0359). The mean of this active small bowel IBD group of 7pg/ml, was less than the mean (14pg/ml) level for the small bowel IBD group as a whole. Patients with active large bowel disease gave a range of 1-184pg/ml and a median of 41pg/ml which was significantly higher than controls p=0.0016 (Table 13B:1C).

There was no significant difference in the TNF levels between controls and the inactive disease groups (Table 13B:1D). There was no significant difference either between disease groups (Table 13B:1E).

COMMENT

Both small bowel and large bowel IBD groups had higher TNF levels than controls showing, as before, that high lavage TNF is a feature of inflammatory bowel disease. And the lack of significant difference between the inactive group and controls indicates that high levels of TNF are associated with active disease.

However closer analysis of the results shows some interesting phenomenon. Whereas the mean, median as well as levels of significance in patients with large bowel disease are highest with the active disease followed by the levels for the whole group with the exclusively inactive being the lowest, the order for the small bowel group is the reverse. This could be a result of increased transit time allowing for the higher levels of proteolytic enzymes in the lavage of active disease patients to have an effect on the levels as discussed with in chapter 12. It is possible, however, that high levels of TNF may not be a feature of active small bowel Crohn's disease. Murch et al. (1991) also reported significantly higher levels in the serum of patients with colonic disease than in those with small bowel disease despite similarities in disease activity. The children with colonic Crohn's disease also showed a lower current growth rate than those with small bowel disease, in keeping with the possible role of TNF in causing cachexia

(Tracey et al., 1988). Braegger et al. (1992) have also reported low levels of TNF in the stool of patients with active disease confined to the small bowel. The lower values for the patients with active small bowel disease therefore are probably a feature of active small bowel disease and not a result of increased proteolytic degradation. This, as stated in chapter 12 may be resolved by collecting intestinal fluids directly from different segments of the bowel, a technically demanding exercise. The reports by Murch et al.(1991) refers to serum levels, and serum TNF would not be influenced by transit time. Even though the low levels reported in this study are in agreement with the reports of Murch et al. (1991), it would still be better to confirm these results with direct sampling of intestinal secretions as systemic immunity does not always mirror intestinal immune activity (O'Mahony et al., 1991a and see chapter 9).

SECTION 13C

LEVELS OF TNF IN WGLF OF PATIENTS WITH IBD CLASSIFIED AS SMALL BOWEL (SB) CROHN'S , CROHN'S COLITIS (CN) AND ULCERATIVE COLITIS (UC)

The large bowel disease group (colonic Crohn's and ulcerative colitis) were further subdivided into ulcerative colitis and colonic Crohn's subgroups and

compared with controls. These are repetitions of results shown earlier; they have been put together only to facilitate comparison in Tables 13C:1A-1E.

The TNF levels in the patients with colonic Crohn's ranged between <1-217pg/ml with a median of 14pg/ml, this was significantly higher than controls (p=0.0073). The patients with UC had a range of <1-184pg/ml with a median of <1pg/ml significant p=0.042 (Table 13C:1A)

The prevalence of high levels (>10pg/ml) of TNF was analysed in these groups. As stated before, out of 15 of the patients with small bowel Crohn's disease 4 had high levels, out of 10 patients with colonic Crohn's disease 5 had high values, and out 23 patients with ulcerative colitis 8 had high values. All the values for the three groups were significantly higher than for controls as shown above. Closer examination of the figures, however, shows that, with a mean of 47 and a median of 14 (Table 13C:1A), Crohn's colitis patients had the highest levels of the three groups. When the prevalence was further analysed according to disease activity, 1 out 9 patients with active small bowel disease had a high value, 3 of the 8 with active Crohn's colitis had high values and 7 of the 13 with active ulcerative colitis had high values. The statistical evaluations of the levels on the three active disease subgroups showed that they were all significantly higher than controls (see below). Three of the 6 patients with inactive small bowel disease had

high levels, both the patients with inactive colonic Crohn's had high levels, and 1 out of the 10 with inactive ulcerative colitis had a high level. There was no significant different in the levels of TNF between these inactive subgroups and controls (see below) [Table 13C:1B].

LEVELS OF TNF IN WGLF OF PATIENTS WITH IBD CLASSIFIED AS SMALL BOWEL(SB) CROHN'S, CROHN'S COLITIS (CN) AND ULCERATIVE COLITIS (UC) ACCORDING TO DISEASE ACTIVITY.

The range for active colonic Crohn's was 1-118pg/ml with a median of 5, significantly higher than controls (p=0.0371) [Table 13C:1B and Table 13C:1C]. There were only two patients with inactive colonic colitis and their levels were 20pg/ml and 217pg/ml.

The active UC group gave a range of <1-184pg/ml with a median of 36pg/ml, and this was significant (p=0.0034) compared to controls, the level of significance, as expected, was higher in the active group than for the group as a whole (Table 13C:1C).

There was no significant difference between the TNF levels in WGLF of controls compared to inactive small bowel Crohn's disease group or inactive ulcerative colitis (Table 13C:1D). There was no significant difference between disease groups either (Table 13C:1E)

COMMENT

When the patients were subgrouped into the three categories, small bowel, colonic and ulcerative colitis, the levels of TNF in WGLF were higher for all groups compared to controls. Further analysis showed that the means and median of patients with colonic Crohn's disease or ulcerative colitis were higher than those for small bowel Crohn's. This agrees with other reports (Murch et al., 1991 and Braegger et al., 1992) and may indicate that high TNF levels are a feature of large bowel Crohn's disease. High levels of TNF in lavage were also found in patients with active ulcerative colitis in this study (Table 13C:1B and Table 13C:1C); this probably suggests that high levels of TNF are a feature of large bowel disease whether Crohn's disease or ulcerative colitis.

SECTION 13D

LEVELS OF TNF IN WGLF OF PATIENTS WITH GLUTEN RELATED ENTEROPATHY GROUP

Whole gut lavage fluid was collected from eight patients with gluten-related enteropathy (7 with coeliac disease and one with dermatitis herpetiformis) , and assayed for TNF by ELISA (see chapter 6A). The levels of TNF in this group were compared with those of controls and patients with IBD.

The gluten related group gave WGLF levels of TNF with a range of <1-44, and a median of <1. These levels were not significantly different from either the IBD patients (UC and CD) or controls (Table 13D:1A). There was no significant difference either when their levels of TNF in WGLF were compared with the levels in patients with active ulcerative colitis or Crohn's disease (Table 13D:1A).

It must be restated that these were patients in remission, on long term gluten free diet and therefore may not represent the true picture with active disease. Whereas their mean level of TNF in WGLF (6pg/ml) were higher than controls (<1pg/ml) they were much lower than the mean levels for ulcerative colitis (28pg/ml) or Crohn's disease (26pg/ml) [Table 13D:1A]. A study of active gluten-related enteropathy is needed to ascertain

the levels and possible role of TNF in gluten related enteropathy .

SECTION 13E

CORRELATIONS

THE RELATIONSHIP BETWEEN DISEASE ACTIVITY (WGLF total IgG) AND GUT LAVAGE TNF

Levels of TNF in WGLF were correlated with levels total IgG in WGLF (see chapter 7) to ascertain whether TNF levels correlated with disease activity.

There was no strong correlation between WGLF IgG for all the groups, controls, ulcerative colitis and Crohn's disease regardless of disease activity (Table 13E:1).

However when Crohn's disease patients were divided into regional involvement, the Crohn's proctitis group showed a borderline significant positive correlation between WGLF IgG and TNF ($r=0.815$, $p=0.048$)[Table 13E:2A]. In the other subgroups active and inactive small bowel, ileocolonic and colonic TNF did not have any strong correlation with gut lavage IgG [Tables 13E:2B-C).

There was no strong correlation between TNF and WGLF IgG for any of the ulcerative colitis groups regardless of the disease activity or distribution (Tables 13E:2A-C).

THE RELATIONSHIP BETWEEN WGLF sIL-2R, SERUM sIL-2R AND WGLF TNF

Levels of TNF in WGLF were correlated with levels of sIL-2R in WGLF and in serum in controls and all the IBD patient subgroups.

There was no strong correlation between serum sIL-2R, whole gut lavage sIL-2R and gut lavage TNF for all Crohn's disease patients and ulcerative colitis regardless of disease activity (Table 13E:3A-C). There was no correlation either when Crohn's disease patients were further subdivided according to regional involvement and disease activity (Table 13E:4A-C).

A similar analysis of patients with ulcerative colitis clearly showed that there was no relationship between the levels of TNF in WGLF and sIL-2R in WGLF or serum (Table 13E:5A-C). This probably suggests that these three measurements mirror different aspects of immune activity. These aspects are discussed later as part of the general comment of this chapter and in chapter 14, as part of the general discussion.

COMMENT

These results show that there is no strong correlation between disease activity as measured by WGLF total IgG and lavage TNF levels. The strong correlation between

disease activity (WGLF total IgG) and TNF levels in WGLF for the patients with Crohn's proctitis is of borderline significance. This level of significance at 1:20 could be expected in view of the many statistical evaluations made. On the otherhand this group showed higher levels of TNF in WGLF (Table 13A:2A) which were much higher than controls especially in the patients with active disease (p=0.0022) [Table 13A:2C]. In addition only the patients with active Crohn's proctitis had high levels on evaluation of the prevalence and not the 2 of the six with inactive disease (Table 13A:2B). Therefore though not many patients with Crohn's proctitis were studied (6), and the level of significance with regard to correlation with disease activity is borderline, it is possible that TNF plays a more prominent role in Crohn's proctitis, than in other disease distributions. A high TNF in a patient with proctitis may be a feature of Crohn's proctitis as the levels of TNF in WGLF of patients with ulcerative proctitis were not higher than the levels for controls (Table 13A:3A). The relationship between disease activity and levels of TNF in WGLF of Crohn's proctitis and the possibility that TNF in lavage may aid distinction between Crohn's proctitis and ulcerative proctitis could be resolved with more numbers studied.

The lack of correlation between sIL-2R in WGLF and lavage TNF despite the fact that they are both raised in Crohn's

disease may be a result of the differences in the predominant sources of these factors. TNF is mainly of macrophage origin while sIL-2R is mainly a product of T cells. Whereas the latter is involved mainly in the early inductive phase (Smith et al., 1979) of the immune response the former is largely involved in effector mechanisms (Tracey et al., 1988; Beutler and Cerami, 1988).

SECTION 13F

ELEMENTAL DIET AND WHOLE GUT LAVAGE TNF

STUDY POPULATION

Eleven patients were prescribed elemental diet in the management of their inflammatory bowel disease for purely clinical reasons. Of the eleven patients, 10 had Crohn's disease, one had ulcerative pancolitis. One patient with coeliac disease was also studied. Whole gut lavage fluid and serum were collected before and after 7 or more days on an exclusive elemental diet and assayed for levels of TNF. At the commencement of the diet all the IBD patients had active disease on global clinical assessment and 9 also by their lavage total IgG (Table 13E).

The disease distribution in the patients with Crohn's disease was as follows; ileal disease (4), colitis (3), ileocolonic (2), and one with both perianal and rectal involvement. The patient with ulcerative colitis had pancolitis.

Drug treatment within three weeks of commencement of the diet was as follows; no drug treatment at all (1), 5-amino salicylic acid only (5), prednisolone only (1), 5-amino salicylate and prednisone (2) and prednisolone and colifoam (1). The patient with ulcerative colitis had been on a combination of 5-amino-salicylate, oral

prednisolone and prednisolone enema and the coeliac disease patient had been on a gluten free diet and no other medication.

All the nutritional requirements were provided for by elemental diet supplying an average of 2000kcal per day. No other food supplements were allowed.

RESULTS

The levels of TNF in WGLF for specimens taken before and while on elemental diet are shown with patient data (Table 13F).

On global assessment 3 of the IBD patients improved, 4 showed no change and four deteriorated.

Their results are plotted graph 13F. This shows that the three patients who responded to elemental diet therapy all started with low levels of TNF in their WGLF (<1 undetectable) while the patients whose clinical condition deteriorated had high levels initially (above 10, as described in earlier sections of this chapter). The levels of TNF in WGLF of the responders remained undetectable in the subsequent specimens. In two of the patients whose clinical condition deteriorated, the TNF level fell to undetectable levels, but one of the group showed a rise in TNF level and in the fourth member of the group (SJ), there was a minimal fall with the levels remaining still high (SJ). Three of this group underwent

laparatomy leading to resections of part of their intestines as they did not respond to medical treatment. The group of 4 whose condition remained static began with TNF levels higher than the responders but less than the group that deteriorated except in one (EM) where the level was <1pg/ml.

COMMENT

Though the numbers are small and the response rate is not as high as reported in literature for reasons given in chapter 12, a pattern again emerges. Invariably all the three responders had undetectable levels of TNF in WGLF. The patients with the highest levels of TNF in WGLF did the worst. This is discussed further in the final discussion (chapter 14).

COELIAC DISEASE AND ELEMENTAL DIET

The patient with coeliac disease had already been on a gluten free diet and her initial lavage TNF was <1pg/ml and rose to 44pg/ml while on elemental diet. There was little change in her clinical condition and not much can be inferred from this single case. This patient is not discussed any further. As for the study of sIL-2R more numbers with acute coeliac disease cases need to be studied.

GENERAL COMMENT

A few observations can be made from the study of TNF in WGLF. The pattern of levels of TNF in disease and controls has been elucidated. The results show that patients with IBD have higher levels of TNF in their WGLF than controls. This is in agreement with the few studies that have looked at TNF levels in serum (see chapter 4B) and the one study that involved measuring TNF levels in stool (Braegger et al., 1992).

Further subdivisions of the disease groups has shown that high levels of TNF in WGLF were accounted for mainly by the patients with active disease (Table 13A:1D).

The median and means were higher for colonic Crohn's disease than for patients with small bowel Crohn's disease as were the levels of significance compared to controls (Table 13A:2A). This is in agreement with the reports of Braegger et al. (1992) who observed that in children with Crohn's disease, which usually involves the colon in this age group, the lowest levels of faecal TNF were recorded in the patient with exclusive ileal disease. Similar observations had been made by Murch et al. (1991) in serum studies.

Patients with ulcerative colitis had significantly higher levels of TNF in their WGLF than controls ($p=0.042$; Table 13A:1B) and the level of significance compared to controls was higher for patients with active ulcerative

colitis (p=0.0034; Table 13A:1D). These observation suggest that high levels of TNF are associated with colonic disease and whether ulcerative colitis or Crohn's colitis. High levels of TNF in ulcerative colitis are probably largely from macrophages in keeping with the finding by Choy et al. (1991) who reported that activated CD25+ cells in UC were of the macrophage dendritic type while in Crohn's colitis this marker (CD25+) of activity was associated with T cells. Macrophages are the main producers of TNF.

The levels of TNF did not correspond with disease activity as measured by whole gut lavage fluid (chapter 7). This is probably because the two substances, whole gut lavage total IgG and TNF are involved in different processes, though they are both raised in active disease. Tumour necrosis factor is involved in the effector and inductive mechanism of the inflammatory response and while lavage total IgG is predominantly a leak from serum (see chapter 7).

The source of the TNF found in whole gut lavage fluid is yet to be fully determined. It could be secreted by macrophages and neutrophils that have leaked from systemic circulation into the bowel lumen in active disease (Saverymuttu et al., 1985b and 1985c) or it may be predominantly of intestinal mucosa origin such as from paneth cell (Kesha et al., 1990) mast cells (Steffen et al., 1989) and indeed T cells (Sherry and

Cerami, 1988). This question is being addressed by other workers in our team.

The group with CD proctitis appears to be different from the patients with CD as a whole. These patients had high individual levels of TNF in WGLF, and in those with active disease the levels were higher. As this did not apply to patients with UC proctitis it is worth following up to see if it would be a distinguishing feature for this group.

The findings in the elemental diet study though involving only 11 patients, are in keeping with previous observations as regards response to the diet. There have been reports that elemental diets were not effective in Crohn's colitis and ulcerative colitis, in this study these two groups had higher levels of TNF compared to controls than small bowel Crohn's. Three patients who responded to elemental diet in this study, two had small bowel disease and one had pancolitis, one of the common denominators was that they all had very low (undetectable) levels of TNF in their WGLF. Larger reviews have shown that even patients with Crohn's colitis do respond to elemental diet (Teahon et al., 1991). These results therefore would tend to explain these findings. In that in keeping with the experience of Crohn's colitis and ulcerative colitis (Endo et al., 1985) being less responsive, in some studies, to elemental diet it is these groups that has high TNF

levels. However as not all UC or Crohn's colitis patients have high levels of TNF, there will be some that will respond to elemental diet.

The physiological or pathological role of TNF in the IBD gut is still an area of intense research. My hypothesis with regard to elemental diet and TNF is that there is always TNF in the intestine which saves a physiological role in inflammatory activity (Bendtzen, 1988 and Bendtzen et al., 1989). This 'regular' TNF is kept under control by homeostatic mechanisms such as the negative feedback exerted by interleukin 4, (Bello-Fernandes et al., 1991), natural antibodies and inhibitors (Seckinger, Isaaz and Dayer, 1989). The patients with the highest levels are the ones in whom regulatory mechanisms have failed and the effector arm (including TNF) continues to operate at higher level without any link to its specific trigger. This non specific inflammatory reaction would not respond to the withdrawal of the triggering factors such as antigen.

SUMMARY

Tumour necrosis factor levels are higher in patients with IBD compared to controls.

These studies suggest that TNF is more associated with colonic disease than small bowel disease. Patients with active UC have the highest levels even significantly

higher than patients with inactive UC (Table 13A:1F). Patients who responded to elemental diet had low (undetectable) levels of TNF while those who deteriorated had high levels. This, if confirmed, would be a useful marker for patient management decisions in inflammatory bowel disease.

APPENDIX FOR CHAPTER 13

TABLE 13:1A STUDY POPULATION

	CROHN'S DISEASE	ULCERATIVE COLITIS	CONTROLS
NUMBERS	45	24	20
SEX M:F	24:21	10:14	7:13
AGE			
MEDIAN	44	38	46
RANGE	14-83	22-79	21-85

DISEASE DISTRIBUTION

CROHN'S DISEASE		ULCERATIVE COLITIS	
Jejunal	4	Rectum only	8
Terminal ileum	11	Left sided	3
Ileo-colonic	13	Pancolitis	11
Colonic	10	Pouchitis	1
Rectal	5	Microscopic	1
Perianal	1	14 Active and 10 inactive	
Microscopic	1		

28 Active and 17 inactive

10 Resections

TABLE 13:1A CONTINUED

CONTROLS	
Polyps	2
Constipation	5
Abdominal pain (unexplained)	8
Hospital workers	3
Diarrhoea (unexplained)	1
Duodenal ulcer	1

DRUG TREATMENT

CROHN'S DISEASE		ULCERATIVE COLITIS	
No drugs	12	No Drugs	7
Prednisolone only (Pred)	10	PRED, ASA and CFM	3
Sulphasalazine (SLZ)	13	ASA and CFM	2
5-amino salicylates only (ASA)	0	ASA only	6
SLZ and Pred	7	ASA and Pred	3
Pred, ASA and colifoam (CFM)	1	ASA,CFM and Azathioprine	1
SLZ, PRED and CFM	1	ASA,Pred and Cyclosporin	1
Pred and CFM	1	CFM only	1

TABLE 13A:1B LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH CROHN'S DISEASE, ULCERATIVE COLITIS AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
UC	23	<1-184	28	1	0.042
CD	45	<1-217	26	2	0.0064

p = Probability that the difference is a chance finding.

TABLE 13A:1C THE FREQUENCY OF HIGH LEVELS OF TNF IN THE WGLF OF PATIENTS WITH ULCERATIVE COLITIS, CROHN'S DISEASE AND CONTROLS. TNF levels above 10pg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>10	N	>10	N	>10
CONTROL	(20)	1		NA		NA
UC	(23)	8*	(13)	7***	(10)	1
CD	(45)	16**	(28)	9**	(17)	7

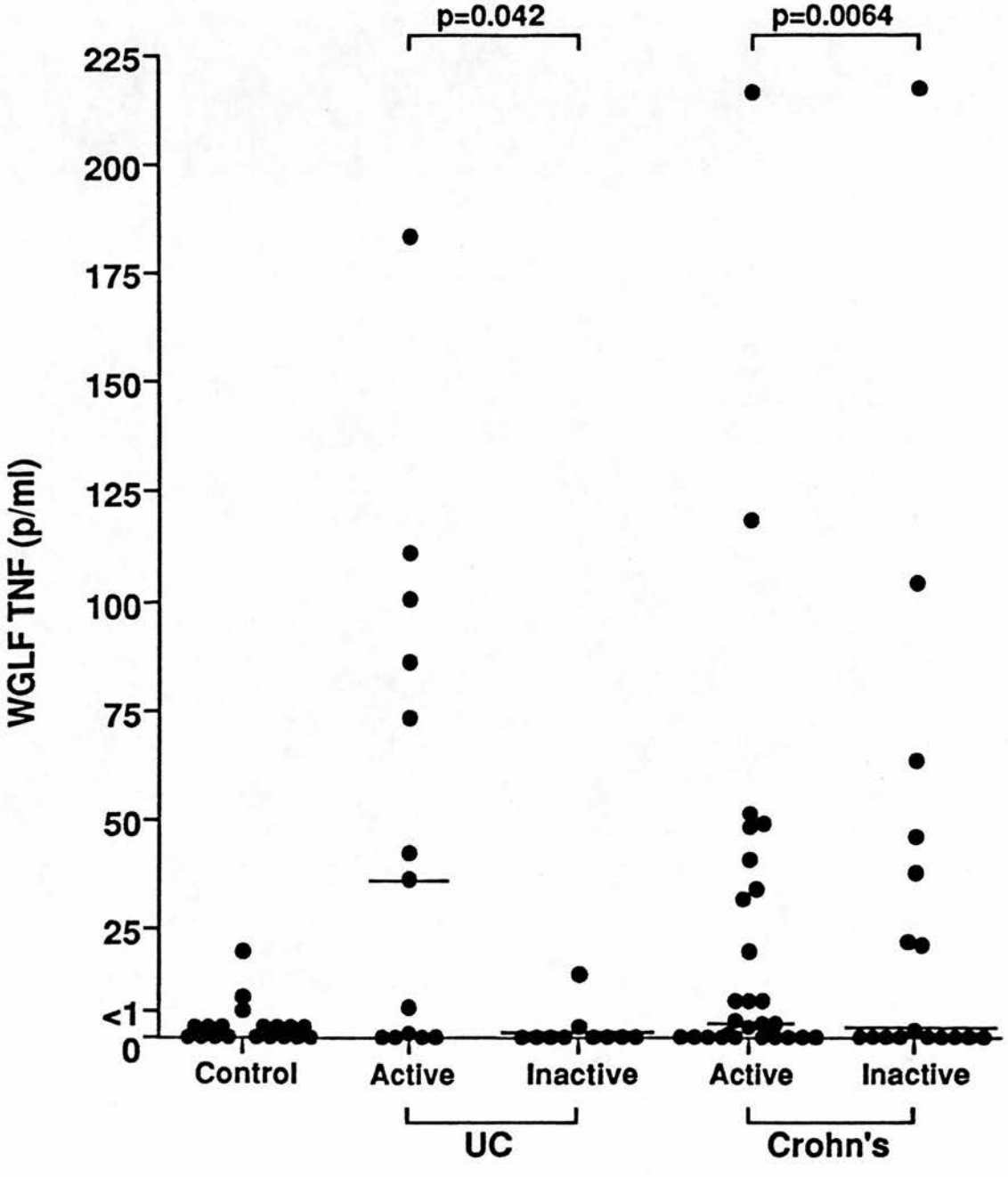
* p < 0.05 ** p < 0.01 *** p < 0.005

NA = Not applicable

N = Total numbers studied in group

Graph 13:1

WGLF TUMOUR NECROSIS FACTOR (p/ml) IN CONTROLS, ULCERATIVE COLITIS (UC) AND CROHN'S DISEASE



Disease groups on the x axis plotted against their levels of tumour necrosis factor on the y axis. Both ulcerative colitis (p=0.042) and Crohn's disease (p=0.0064) had significantly higher values than controls.

TABLE 13A:1D LEVELS OF TNF(pg/ml) IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE, ACTIVE ULCERATIVE COLITIS AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
aUC	13	<1-184	49	36	0.0034
aCD	28	<1-217	23	3	0.0072

p = Probability that the difference is a chance finding.

TABLE 13A:1E LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH INACTIVE CROHN'S DISEASE, INACTIVE ULCERATIVE COLITIS AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
inaUC	10	<1-14	2	<1	0.8776
inaCRO	17	<1-217	30	<1	0.0549

p = Probability that the difference is a chance finding.

TABLE 13A:1F COMPARISON OF LEVELS OF TNF IN WGLF BETWEEN DISEASE GROUPS.Statistical comparisons by Mann-Whitney (p values).

DISEASE GROUPS	p
UC vs CD	0.8458
aUC vs aCD	0.2125
inaUC vs inaCRO	0.1524
aUC vs inaUC	0.0200
aCD vs inaCRO	0.8791

p = Probability that the difference is a chance finding.

TABLE 13A:2A LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	15	<1-63	14	1	0.0196
ILC	13	<1-104	14	<1	0.2240
CN	10	<1-217	47	14	0.0073
PR	6	<1-217	26	26	0.0288

SB - Small bowel Crohn's disease CN - Colonic Crohn's disease

ILC - Ileocolonic Crohn's disease PR - Crohn's Proctitis

TABLE 13A:2B THE FREQUENCY OF HIGH LEVELS OF TNF IN THE WGLF OF CROHN'S DISEASE PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT. TNF levels above 10pg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>10	N	>10	N	>10
CONTROL	(20)	1		NA		NA
SB	(15)	4*	(9)	1*	(6)	3
ILC	(13)	3	(6)	1	(7)	2
CN	(10)	5**	(8)	3*	(2)	2
PR	(6)	4*	(4)	4**	(2)	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

NA = Not applicable

N = Total numbers studied in group

TABLE 13A:2C LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	9	<1-41	7	1	0.0359
ILC	6	<1-51	10	1.5	0.4652
CN	8	<1-118	28	5	0.0371
PR	4	20-217	76	32	0.0022

p = Probability that the difference is a chance finding.

TABLE 13A:2D LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH INACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	6	<1-63	19	1	0.1281
ILC	7	<1-104	14	<1	0.2567
CN	2	NA	NA	NA	NA
PR	2	NA	NA	NA	NA

NA = Not applicable

p = Probability that the difference is a chance finding.

TABLE 13A:3A LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-19	1	<1	
PAN	9	<1-183	40	4	0.0235
PR	8	<1-100	18	<1	0.4924

p = Probability that the difference is a chance finding.

TABLE 13A:3B THE FREQUENCY OF HIGH LEVELS OF TNF IN THE WGLF OF ULCERATIVE COLITIS PATIENTS CLASSIFIED BY REGIONAL INVOLVEMENT. TNF levels above 10pg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>10	N	>10	N	>10
CONTROL	20	1		NA		NA
PAN	9	4*	6	3**	4	1
LS	3	1	2	1	1	0
PR	8	2	4	2	4	0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

p = Probability that the difference is a chance finding.

TABLE 13A:3C LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH ACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-19	1	<1	
PAN	6	<1-183	65	46	0.0056
LS	2	NA	NA	NA	NA
PR	4	<1-100	35	21	0.1752

NA = Not applicable

p = Probability that the difference is a chance finding.

TABLE 13A:3D LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH INACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-19	1	<1	
PAN	4	<1-14	4	<1	0.6701
LS	1	NA	NA	NA	NA
PR	4	<1-<1	<1	<1	NA

NA = Not applicable

p = Probability that the difference is a chance finding.

TABLE 13B:1A LEVELS OF TNF (pg/ml) IN WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) AND LARGE BOWEL (LB) INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	15	<1-63	14	1	0.0196
LB	33	<1-217	34	2	0.0078

p = Probability that the difference is a chance finding.

TABLE 13B:1B THE FREQUENCY OF HIGH LEVELS OF TNF IN THE WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL AND LARGE BOWEL INVOLVEMENT. TNF levels above 10pg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>10	N	>10	N	>10
CONTROL	(20)	1		NA		NA
SB	(15)	4*	(9)	1*	(6)	3
LB	(33)	13**	(21)	10***	(12)	3

NA = Not applicable

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

p = Probability that the difference is a chance finding.

N = Total numbers in studied in the group

TABLE 13B:1C LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH ACTIVE IBD CLASSIFIED AS SMALL BOWEL AND LARGE BOWEL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	9	<1-41	7	1	0.0359
LB	21	<1-184	41	8	0.0016

p = Probability that the difference is a chance finding.

TABLE 13B:1D LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH INACTIVE IBD CLASSIFIED AS SMALL BOWEL AND LARGE BOWEL INVOLVEMENT.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	6	<1-63	25	1	0.1281
LB	12	<1-217	21	<1	0.3603

p = Probability that the difference is a chance finding.

TABLE 13B:1E COMPARISONS IN LEVELS OF TNF IN WGLF BETWEEN SMALL BOWEL AND LARGE BOWEL INVOLVEMENT. Statistical comparisons by Mann-Whitney (p values).

	ALL	ACT	INACT
SB vs LB	0.6727	0.2133	0.4260

p = Probability that the difference is a chance finding.

TABLE 13C:1A LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH IBD CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	15	<1-63	14	1	0.0196
CN	10	<1-217	47	14	0.0073
UC	23	<1-184	28	<1	0.042

p = Probability that the difference is a chance finding.

TABLE 13C:1B THE FREQUENCY OF HIGH LEVELS OF TNF IN THE WGLF OF IBD PATIENTS CLASSIFIED AS SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). TNF levels above 10pg/ml.

	ALL		ACTIVE		INACTIVE	
	N	>10	N	>10	N	>10
CONTROL	(20)	1		NA		NA
SB	(15)	4*	(9)	1*	(6)	3
CN	(10)	5**	(8)	3*	(2)	2
UC	(23)	8*	(13)	7**	(10)	1

NA = Not applicable

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.005$

N = Total numbers in studied in the group

TABLE 13C:1C LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH ACTIVE IBD CLASSIFIED AS ACTIVE SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	9	<1-41	7	1	0.0359
CN	8	<1-118	28	5	0.0371
UC	13	<1-184	49	36	0.0034

p = Probability that the difference is a chance finding.

TABLE 13C:1D LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH INACTIVE IBD CLASSIFIED AS INACTIVE SMALL BOWEL (SB) CROHN'S DISEASE, INACTIVE COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC).

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	
SB	6	<1-63	25	1	0.1281
CN	2	NA	NA	NA	NA
UC	10	<1-14	2	<1	0.8776

NA = Not applicable

p = Probability that the difference is a chance finding.

TABLE 13C:1E COMPARISONS OF LEVELS OF TNF IN WGLF BETWEEN SMALL BOWEL (SB) CROHN'S DISEASE, COLONIC (CN) CROHN'S DISEASE AND ULCERATIVE COLITIS (UC). Statistical comparisons by Mann-Whitney (p values).

	SB vs UC	SB vs CN	UC vs CN
ALL	0.9524	0.2330	0.2814
ACT	0.2045	0.4414	0.6378
INACT	0.2123	NA	NA

NA = not applicable

p = Probability that the difference is a chance finding.

TABLE 13D:1A LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH GLUTEN RELATED ENTEROPATHY COMPARED WITH IBD AND CONTROLS.

	N	RANGE	MEAN	MEDIAN	p
CONTROL	20	<1-20	1	<1	0.6841
UC	23	<1-184	28	<1	0.1604
CD	45	<1-217	26	2	0.2887
GRE	8	<1-44	6	<1	

p = Probability that the difference is a chance finding.

GRE = Gluten related enteropathy

TABLE 13D:1B LEVELS OF TNF (pg/ml) IN WGLF OF PATIENTS WITH GLUTEN RELATED ENTEROPATHY COMPARED WITH ACTIVE IBD PATIENTS.

	N	RANGE	MEAN	MEDIAN	p
aUC	13	<1-184	49	36	0.1429
aCD	28	<1-217	23	3	0.0550
GRE	8	<1-44	6	<1	

GRE = Gluten related enteropathy

p = Probability that the difference is a chance finding.

CORRELATIONS

TABLE 13E:1 CORRELATIONS BETWEEN DISEASE ACTIVITY (WGLF total IgG) AND TNF LEVELS IN WGLF

GROUP	CROHN'S			UC		
	N	r	p	N	r	p
ALL	45	0.060	0.694	23	0.493	0.067
ACT	28	0.163	0.406	13	0.225	0.459
INACT	17	-0.090	0.731	10	-0.242	0.501

p = the probability that the correlation is a chance finding.

TABLE 13E:2A CORRELATIONS BETWEEN LEVELS OF TNF IN WGLF AND DISEASE ACTIVITY (WGLF total IgG) BY REGIONAL INVOLVEMENT OF IBD.

GROUP	CROHN'S DISEASE			GROUP	ULCERATIVE COLITIS		
	N	r	p		N	r	p
SB	15	-0.059	0.834	PAN	10	0.525	0.120
ILC	13	-0.156	0.611	PRO	8	0.635	0.091
CN	10	-0.219	0.543				
PR	6	0.815	0.048				

TABLE 13E:2B CORRELATIONS BETWEEN LEVELS OF TNF IN WGLF AND DISEASE ACTIVITY (WGLF total IgG) BY REGIONAL INVOLVEMENT OF ACTIVE IBD.

GROUP	CROHN'S DISEASE			GROUP	ULCERATIVE COLITIS		
	N	r	p		N	r	p
SB	9	0.554	0.122	PAN	7	0.307	0.554
ILC	6	-0.333	0.519	PRO	4	0.484	0.516
CN	8	0.130	0.759				
PR	6	0.941	0.059				

P = the probability that the correlation is a chance finding

TABLE 13E:2C CORRELATIONS BETWEEN LEVELS OF TNF IN WGLF AND DISEASE ACTIVITY (WGLF total IgG) BY REGIONAL INVOLVEMENT OF INACTIVE IBD.

GROUP	CROHN'S DISEASE			GROUP	ULCERATIVE COLITIS		
	N	r	p		N	r	p
SB	6	0.001	0.998	PAN	4	-0.396	0.604
IL	7	0.615	0.141	PRO	4	NA	NA
CN	2	NA	NA				
PR	2	NA	NA				

P = the probability that the correlation is a chance finding

TABLE 13E:3A CORRELATIONS BETWEEN sIL-2R in SERUM OR WGLF AND LEVELS OF TNF IN WGLF OF PATIENTS WITH ULCERATIVE COLITIS, CROHN'S DISEASE AND CONTROLS.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
CONTROL	20	0.602	0.005	11	0.134	0.695
CRO	45	-0.183	0.230	38	0.223	0.178
UC	23	0.259	0.232	19	0.065	0.791

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

TABLE 13E:3B CORRELATIONS BETWEEN LEVELS OF sIL-2R IN SERUM OR WGLF AND LEVELS OF TNF IN WGLF OF PATIENTS WITH ACTIVE IBD.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
CRO	28	-0.184	0.350	25	0.281	0.173
UC	13	0.077	0.802	11	-0.077	0.823

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13E:3C CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND LEVELS OF TNF IN PATIENTS WITH INACTIVE IBD.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
CRO	17	-0.179	0.491	13	-0.293	0.331
UC	10	0.095	0.794	8	-0.080	0.851

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13E:4A CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND THE LEVELS OF TNF IN CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
SB	15	-0.054	0.848	13	-0.087	0.778
ILC	13	-0.225	0.461	9	0.524	0.147
CN	7	-0.308	0.386	4	-0.249	0.518
PR	6	-0.174	0.741	6	0.848	0.033

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13E:4B CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND LEVELS OF TNF IN WGLF OF PATIENTS WITH ACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
SB	9	-0.029	0.941	8	-0.509	0.198
ILC	6	-0.223	0.671	5	0.797	0.107
CN	8	-0.381	0.352	7	-0.097	0.835
PR	4	-0.446	0.554	4	0.814	0.186

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

TABLE 13E:4C CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND LEVELS OF TNF IN WGLF OF PATIENTS WITH INACTIVE CROHN'S DISEASE CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R		TNF vs BIL2R		
	N	r	p	N	r	p
SB	6	-0.184	0.727	5	0.628	0.256
ILC	7	-0.218	0.630	4	-0.664	0.336
CN	2	NA	NA	2	NA	NA
PR	2	NA	NA	2	NA	NA

NA = Not applicable

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13E:5A CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND THE LEVELS OF TNF IN ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
PAN	10	0.170	0.638	9	0.161	0.680
PRO	8	0.552	0.156	6	-0.256	0.624

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

TABLE 13E:5B CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND THE LEVELS OF TNF IN ACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R			TNF vs BIL2R		
	N	r	p	N	r	p
PAN	6	0.133	0.801	6	-0.113	0.831
PR	4	0.305	0.695	3	-0.603	0.588

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13E:5C CORRELATIONS BETWEEN sIL-2R IN WGLF OR SERUM AND THE LEVELS OF TNF IN INACTIVE ULCERATIVE COLITIS CLASSIFIED BY REGIONAL INVOLVEMENT.

GROUP	TNF vs LIL2R		TNF vs BIL2R		
	N	r	p	N	r	p
PAN	4	0.097	0.903	4	0.881	0.314
PRO	4	NA	NA	3	NA	NA

NA = Not applicable

BIL2R = sIL-2R in serum

LIL2R = sIL-2R in WGLF

P = the probability that the correlation is a chance finding

TABLE 13F

TUMOUR NECROSIS FACTOR AND ELEMENTAL DIET PATIENT DATA

						CLINICAL DATA																			
NAME	AGE	SEX	DIAG	INDICATION FOR DIET	RESECTIONS	DOD	HB	CRP	ESR	WT	ALB	LTIGG	LTNF			HB	CRP	ESR	WT	ALB	LTIGG	LTNF			
DM	29	F	1	* STEROID FAILURE	NONE	21	14	2.3	37	50	36	64	<1			13	1.5	15	49	38	35	<1			
BS	28	M	UC	* STEROID FAILURE	NONE	10	11	10	90	46	34	120	<1			11	1.5	50	51	34	120	<1			
DS	30	F	1	RECRUDESCENCE OF DISEASE	HEMICOLECTOMY	31	10	3.6	25	42	30	64	35			11	3.7	32	44	31	61	1			
DB	19	M	1	* RECURRENT ACTIVE DISEASE	ILEOCOLECTOMY	21	11	5.5	63	52	36	3	<1			10	1.5	37	52	34	1	<1			
AM	38	F	2	OBSTRUCTIVE SYMPTOMS OF CROHN'S DISEASE	PROCTOCOLECTOMY	21	13	3.6	34	68	36	19	<1			15	1.5	17	68	42	12	20			
EW	25	F	3	RECURRENT ACTIVE DISEASE	NONE	14	11	2.0	83	45	34	133	42			9.5	3.9	80	45	34	131	105			
MI	50	F	2	ACTIVE ILEAL DISEASE	RT HEMICOLECTOMY	21	14	1.5	15	–	31	1	10			13	1.5	11	–	30	1	119			
JM	54	F	3	RECURRENT ACTIVE DISEASE	SIGMOID RESECTION	14	11	10	46	66	32	15	51			10	1.5	42	64	33	12	<1			
CC	16	M	1PA	PERSISTENTLY ACTIVE CROHN'S DISEASE	SUBTOTAL COLECTOMY	14	10	7	25	25	28	47	217			15	1.5	33	25.5	31	19	<1			
SJ	26	M	3	ABDOMINAL PAIN AND DIARRHOEA	NONE	21	14	1.5	33	82	40	2	217			14	1.5	13	83	40	5	190			
EM	51	F	2	SB OBSTRUCTION RELUCTANT TO TAKE STEROIDS	TERMINAL ILEOCTOMY	14	13	11	79	57	40	29	<1			12	16	92	60	40	22	<1			
ES	34	F	COEL	NOT RESPONDING TO GLUTEN FREE DIET	NONE	30	12	–	8	40	43	3	<1			12	1.5	10	41	44	2	44			

← BEFORE ELEMENTAL DIET → ← ON ELEMENTAL DIET →

KEY

1 = ILEAL CROHN'S DISEASE

1PA = ILEAL AND PERIANAL DISEASE

2 = ILEOCOLON CROHN'S DISEASE

3 = COLONIC CROHN'S DISEASE

UC = Ulcerative colitis

COEL = Coeliac disease

DOD = DAYS ON DIET

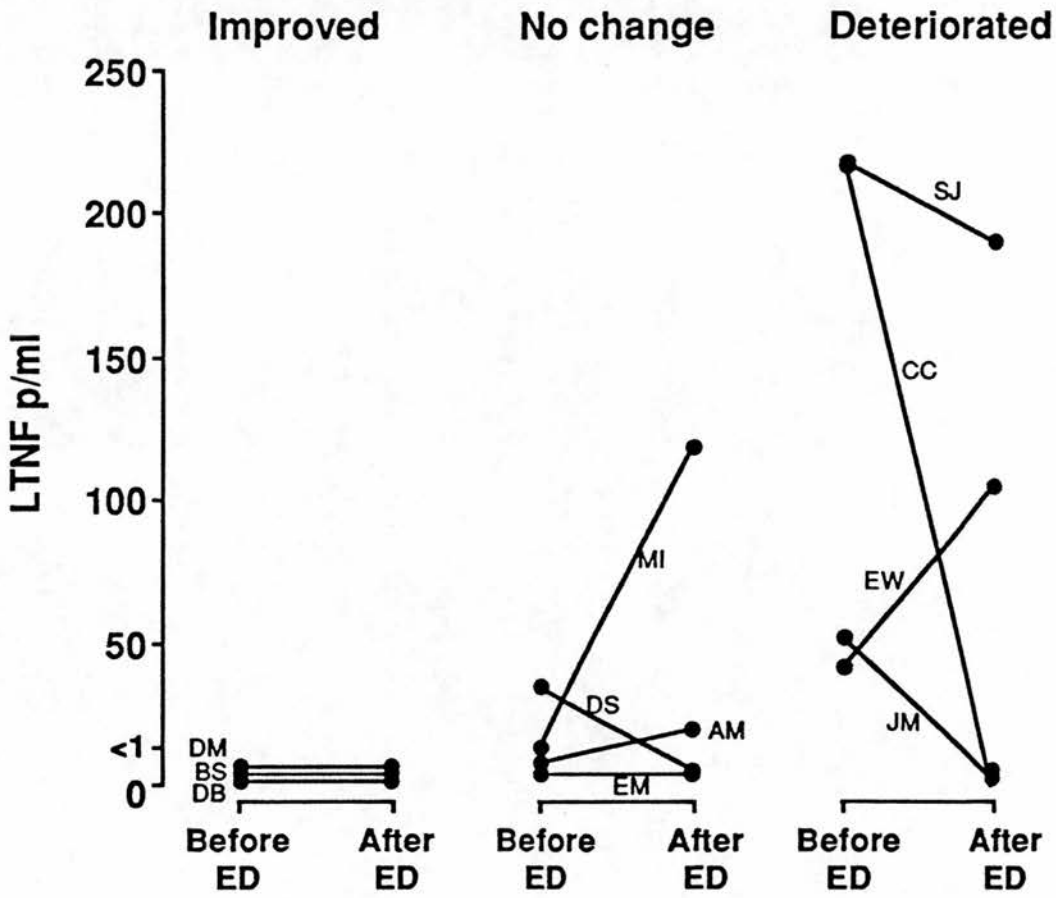
LTNF = LAVAGE TUMOUR NECROSIS FACTOR

LTIGG = LAVAGE TOTAL IgG

* = RESPONDERS

Graph 13F

WHOLE GUT LAVAGE FLUID TUMOUR NECROSIS FACTOR LEVELS AND ELEMENTAL DIET



Levels of tumour necrosis factor in WGLF before and after at least seven days on elemental diet. On the y axis are the levels (p/ml) and the x axis the time span. Patients were grouped according to global clinical response as improved, no change or deteriorated.

CHAPTER FOURTEEN:

DISCUSSION

DISCUSSION AND CONCLUSION

The aim of this thesis was to study the biology of immune responses to food antigens in inflammatory bowel disease (IBD). There have been reports of raised serum levels of antibodies to food antigens in patients with inflammatory bowel disease (Taylor and Truelove, 1961; Koninckx et al., 1984), and these were discussed in Chapter 2B. Whereas no single food antigen has been found to be consistently associated with ulcerative colitis or Crohn's disease, as for example is the case with coeliac disease, the exclusion of polypeptides by the use of elemental diets has been found to alleviate clinical symptoms (O'Morain et al., 1980; 1984; Giaffer et al., 1990) and this was discussed in chapter 5. Elemental diets have been found to be beneficial in many other conditions, such as pancreatitis, ischaemic colitis and radiation enteropathy. The mechanisms by which they act in these conditions probably vary. In IBD the mechanism by which elemental diets act remains unknown, but in view of the immunological disturbances that are part of IBD as discussed in chapters 1,2A,4D and by other workers (Broberger and Perlmann, 1962; Mahida et al., 1990; MacDermott and Stenson, 1988;) and shown in this thesis (chapters 7,10,12,13), the involvement of the immune system in their action is probable.

Assessment of disease activity by clinical symptoms and signs can be subjective and makes scientific study difficult. An objective and universal measurement of IBD disease activity was developed as described in Chapter 7. The concentration of IgG in whole gut lavage fluid (WGLF) was found to correlate strongly with Crohn's disease activity index (CDAI) for Crohn's disease and Powell-Tuck index for ulcerative colitis. The IgG in the gut lavage fluid is probably of serum origin.

Defining this marker of disease activity facilitated the objective subgrouping of patients into active and inactive groups. As the total IgG concentration in WGLF correlated with disease activity, it was possible to assess whether the degree of disease activity was linked to cytokine (T cell) activity or the presence of antibodies to food antigens. Further detailed study of the patients was achieved by subdividing patients according to the predominant regions of gut grossly affected as determined by radiology, endoscopy and histopathology.

Measurement of IgA levels in WGLF showed that the levels were increased in patients with IBD. The proportion of secretory IgA to total IgA was diminished in patients with IBD compared to controls (Chapter 8). This could indicate that the secretion of IgA outstrips the capacity for binding cytoplasmic secretory component (Brandtzaeg and Korsud, 1984). As the binding of secretory component

is required for the transport of IgA across the epithelium into the lumen, reduced binding capacity of secretory component is probably not the main factor for the decreased proportions of secretory IgA in IBD patients compared to controls. However the concentration of non-secretory IgA in IBD patients increased with disease activity and it correlated with whole gut lavage IgG ($r=0.241$ $p<0.0001$) [Table 8:11]. Thus I suggest that much of the non-secretory IgA in WGLF is serum derived the same as for whole gut lavage IgG, reflecting a protein losing enteropathy. Further studies of the subclasses of IgA in WGLF of patients with active disease will confirm the source of luminal IgA. An increased proportion of IgA1 in the WGLF of patients with active disease compared to controls would support the possibility that serum as the source of non-secretory IgA.

In chapter 9 total immunoglobulins and levels of antibodies to three dietary antigens were measured in the intestinal secretions (WGLF and parotid saliva) and sera of IBD patients and controls. The three ubiquitous food antigens studied were ovalbumin, ß-lactoglobulin and gliadin. The patients were studied according to their disease activity (based on global clinical assessment and the levels of IgG in WGLF) and the regional distribution of their disease.

The results were as follows.

No differences between disease groups and controls were detected in serum. By definition patients with active disease had higher levels of IgG in WGLF than controls or patients with inactive disease. There were no significant differences between disease groups and controls in WGLF total IgA.

Total IgM in WGLF was higher for patients with active IBD than controls. IgM is also raised in other immune based diseases such as coeliac disease (O'Mahony et al.,1991a). This raised IgM could be due to the fact that IgM, being the immunoglobulin class of the primary immune response, increases non-specifically whenever there is increased drive to B cell activity; however it is likely that this is coupled with an aberration in the maturation process. It is therefore conceivable that the more active disease would be associated with a higher degree of immune aberration and therefore a higher concentration of IgM as was seen in this study. The higher levels of IgM in the whole gut lavage fluid of patients with active inflammatory bowel disease would therefore reflect this non-specific aberration in the maturation process.

A study of antibody levels to the three dietary antigens gave significant differences between IBD patients and controls in WGLF and not in serum. Patients with active Crohn's disease had higher levels of food antibodies to ovalbumin and gliadin in the IgA ($p=0.0269$ and $p=0.0073$)

and IgM ($p=0.0140$ and $p=0.0269$) classes. There was no difference in food antibody levels between patients with ulcerative colitis and controls or Crohn's disease patients. This puts the patients with ulcerative colitis in an intermediate group, in terms of immune upregulation to food antigens, between active Crohn's disease (greater immune upregulation) and controls. Two possible explanations for this are that contact between antigen and immune cells in the lamina propria may be easier in Crohn's disease patients due to the transmural nature of their disease or that Crohn's disease patients have intrinsically an upregulated underlying gut immune activity.

Only WGLF and not serum showed significant differences between controls and disease groups for total immunoglobulins and food antibody levels. There was no correlation between levels of antibodies in serum and levels in WGLF. This is additional evidence that systemic immunity and mucosal immunity are independent.

Having established that Crohn's disease patients were the hyperimmune group, the experiments described in chapter 10 measured the effect of elemental diets on these antibodies in serum and intestinal secretions (WGLF and saliva).

Baseline studies showed that parotid saliva total immunoglobulins were mainly in the IgA class. The levels of total IgA in saliva in each subject varied from day to

day within a narrow range for each subject (Graph 10:2B). This meant that large changes in these levels resulting from, for example, treatment with antigen withdrawal could be studied. There was no correlation between total serum IgA and saliva IgA. Smoking has been reported to reduce saliva IgA (Barton et al., 1990) but this relationship was not confirmed in this study probably because the patients smoked less than 20 cigarettes a day and a few consumed alcohol as well which is known to increase IgA levels.

The antibodies specific for the three antigens (gliadin, ß-lactoglobulin and ovalbumin) in saliva were also predominantly in the IgA class, and their levels did not correlate with the serum IgA antibody levels.

When patients were prescribed elemental diet, there was no significant change in serum or saliva total immunoglobulin levels regardless of the clinical outcome (i.e. whether or not the diet altered the symptoms). The same applied to the levels of antibodies to the three food antigens, even when patients were divided into those who responded to elemental diet and those who did not respond.

Studies of WGLF immunoglobulins and food antibodies with respect to elemental diet also showed that there were no significant change in their levels that could be related to disease response or lack of it. Total IgG in WGLF fell (Table 10D:2 and Table 10D:2A) in keeping with the

general improvement in the clinical condition of patients, most probably as a result of reduced leakage of serum IgG into the intestinal lumen. The more sensitive ELISPOT assay which involved enumeration of cells secreting particular antibodies in peripheral blood was performed to see if there were changes that were too early for detection by ordinary ELISA. There was no significant change in the number of cells secreting antibody to food antigens in patients taking elemental diet regardless of the clinical outcome. This phase of the work therefore showed that modulation of humoral immunity was not a primary mechanism in the action of elemental diets in IBD.

To test whether elemental diet modifies macrophage activity two assays, one indirect and one direct were used. The indirect method was by measuring α-1AP in serum and WGLF. IL-1, IL-6 and TNF are produced in abundance mainly by macrophages, and α-1 acid glycoprotein is produced by the liver as a result of their actions. The possible secretion of α-1AP from the liver via bile was studied by using whole gut lavage fluid. The levels of α-1 acid glycoprotein were higher in both active and inactive disease groups compared to controls. The results in WGLF followed the pattern in serum, this could be because WGLF α-1 acid glycoprotein, despite the contribution from bile was mainly leaking from serum. When α-1 acid glycoprotein was studied with respect to

acute changes as in the response to elemental diet, there was no relationship between disease response and the levels of α-1 acid glycoprotein in serum or WGLF.

Direct measurement of macrophage secretory activity via tumour necrosis factor (TNF) levels in WGLF was more informative. Patients with active Crohn's disease and active ulcerative colitis had significantly higher levels than controls. When patients were further studied according to regional involvement of disease, the patients with Crohn's colitis and ulcerative colitis had relatively higher levels than patients with small bowel involvement (Table 13C:1A-1E). When elemental diet was prescribed, it was the patients with the low levels of TNF who responded. The patients with high levels did not respond to elemental diet. I suggest that high levels of TNF indicate a later, more generalised upregulation of the inflammatory activity not amenable to treatment by specific antigen withdrawal. The finding of high levels of TNF in Crohn's colitis and ulcerative colitis might explain why some workers have found that these patients (Endo et al., 1985) are less responsive to elemental diet.

Soluble interleukin-2 receptor (sIL-2R), the p55 chain of the T-cell receptor, was used as a marker of T cell activity and measured in WGLF and serum. In keeping with previous reports (Mahida et al., 1988; Brynskov and Tvede, 1990) sIL-2R in serum was significantly higher in

patients with active Crohn's disease and active ulcerative colitis compared to controls (Table 12:1A). There was no significant difference in serum sIL-2R levels between inactive disease and controls (Table 12:1D). In whole gut lavage fluid sIL-2R was largely undetectable in controls. There was no significant difference in WGLF sIL-2R between patients with ulcerative colitis and controls ($p = 0.0533$). Crohn's disease patients had significantly higher levels than controls ($p = 0.003$) [Table 12B:1A and Table 12B:1B]. However, both active disease groups (CD and UC) had significantly higher levels than controls (Graph 12B:1). Further subdivisions showed that the patients with small bowel disease had relatively higher levels of sIL-2R than patients with disease confined to the large bowel.

When patients were prescribed elemental diet, the patients that improved had high initial levels of sIL-2R in their lavage fluid which fell with disease improvement. It was these same patients who had low levels of TNF. The patients with low levels of sIL-2R in lavage also had high levels of TNF. I suggest that high sIL-2R in lavage serves not only as a marker of patients with predominantly immune related bowel disease which may be amenable to antigen withdrawal, but also may have been facilitatory in the down regulation of the immune response by elemental diets just as cyclosporin acts in IBD (Brynskov and Tvede, 1990). The mode of action of

cyclosporin involves the blocking of the induction of mRNA for IL-2 in T cells (Granelli-Piperno, Inaba and Steinman, 1984). I would suggest that in the absence of stimulatory antigens sIL-2R may act as a negative feedback, blocking the action of IL-2 by binding with it before it reaches the target cells. Levels of IL-2 were not measured in this study but these have been reported to be increased in patients with active IBD compared to controls (Brynskov and Tvede, 1990).

The primary antigen(s) in inducing or exacerbating IBD is (are) still unknown. However the immune response in IBD is upregulated as shown in this thesis. The levels of antibodies to food antigens were higher in IBD as were the levels of tumour necrosis factor and sIL-2R. With this background of an upregulated immune response it is probable that the immune reaction to any antigens is not finely controlled and directed (Poo, Conrad and Janeway, 1988). This makes it difficult to identify the primary antigen(s) in the immunopathogenesis, as the effector mechanisms would be non-specifically upregulated against a variety of antigens. I propose that food antigens being one of the commonest antigens in the intestinal lumen are non-specifically involved in the exacerbation or induction of the upregulated immune response. Their withdrawal therefore leads to a diminished antigen load and, in patients whose regulatory mechanisms are still intact (such as those with raised sIL-2R), there is

concomitant down regulation of the immune response. In the patients who have raised macrophage activity, the effector mechanisms may have become too non-specific for any specific withdrawal of antigens to be effective.

Food antigens may not be responsible directly for exacerbating IBD but their effect on the gut microbial flora may be the underlying mechanism. For example; in the guinea pigs the presence of Bacteriodes vulgaris is necessary for carrageenan to cause the ulcerative colitis-like mucosal lesions (Onderdonk et al.,1987). If a similar mechanism is involved in human IBD, then a change in the load or strains of the bacteria in the gut may be the mechanism by which elemental diet leads to a down regulation of the immune response. The reports on the effect of elemental diet on bacterial flora are not conclusive (Menge et al., 1985; Crowthen et al., 1976) and discussed in Chapter 5. Menge et al. (1988) found no difference while Crowthen et al. (1973) had reported an increase in enterobacteria and a decrease in enterococci. The effect of elemental diet on gut flora needs to be resolved and work toward resolving this question is needed.

The two markers that were associated with disease response (TNF and sIL-2R) reflect both the regulator and effector ends of the immune response. Tumour necrosis factor is involved in the effector mechanisms while the IL-2 system is mainly involved in the controlling stages

of the immune response. As cytokines seldom act in isolation more data on other cytokines is necessary are in order to get the full picture; for example, IL-4 which may be involved in down-regulating TNF (Bello-Fernandez et al., 1991). Deficiency of IL-4 in patients with raised TNF might therefore result in the failure of down regulation of immune responses in the gut.

In summary this thesis was a study of immune responses to food antigens in IBD. Levels of antibodies to the three food antigens were raised in patients with Crohn's disease compared to controls in whole gut lavage fluid, but not in serum. Serum and WGLF sIL-2R levels were raised in active IBD patients which indicated increased T cell activity compared to controls. The patients who responded to elemental diet had high initial levels of sIL-2R in their WGLF. On the other hand, patients who had raised levels of TNF, a marker of macrophage activity, were not responsive to elemental diet. The possible explanation for these findings have been discussed. The source of the sIL-2R and TNF in WGLF of IBD patients is not fully ascertained. They could both originate from the systemic circulation and reach WGLF by leakage through disease-damaged epithelium, but the lack of correlation between levels of sIL-2R in WGLF and serum suggested independent sources. There is now increasing evidence that both sIL-2R (Mahida et al.,1988; Choy et

al., 1990) and TNF (MacDonald et al.,1990) are largely of local mucosal origin. There may be other cytokines involved. The combination of these cytokines may point towards the type of T cell involved in the down-regulation of IBD immune activity by elemental diet.

The patients defined by high levels of whole gut lavage sIL-2R and low levels of TNF is a cohort that responds to elemental diet. If confirmed, these criteria will contribute towards making the clinical management of IBD patients less empirical. There is also the need to examine how these factors relate to cases which respond to other types of treatment.

The limitation to using these criteria routinely is that the two useful markers TNF and sIL-2R in WGLF are very expensive to assay. However if the usefulness of these assays is proved they may become less expensive or they may be superseded by less expensive tests amenable to routine use.

REFERENCES

Adams, D.O. and Hamilton, T.A. (1987) Molecular transductional mechanisms by which IFN gamma and other signals regulate macrophage development.
Immunol. Rev. 97:5-21

Aggarwal, B.B., Kohr, W.J., Hass, P.E., Moffat, B., Spencer, S.A., Henzel, W.J., Bringman, T.S., Nedwin, G.E., Goeddel, D.V. and Harkins, R.N. (1985) Human tumour necrosis factor. Production, purification, and characterization.
J. Biol. Chem. 260(4):2345-54

Allchin, W. H. (1909) Ulcerative colitis.
Proc. R. Soc. Med. II. Med Sect. ii:59-82

Alstead, E. M., McConnell, J.S., Exley, A. R., Hodgson, H. J. F. and Cohen, J. (1991) Relationship between endotoxin, tumour necrosis factor-α and disease activity in inflammatory bowel disease.
Eur. J. Gastro. and Hepatol. 3:325-328

Alun Jones, V., Dickinson, R.J., Workman, E., Freeman, A. H. and Hunter, J. O. (1985) Crohn's disease: maintenance of remission by diet.
Lancet ii:177-80

Andresen A.F.R. (1925) Gastrointestinal manifestations of food allergy.
Med. J. Rec. 122:271-5

Andresen A.F.R. (1953) Allergic manifestations in the gastrointestinal tract.
Gastroenterology 23:20-35

Archer, L.N.J. (1977) Breakfast and Crohn's disease II.
Br. Med. J. ii:540

Arden, L. A. (1979) Letter to the editor: Revised nomenclature for Antigen Non-specific T cell Proliferation and helper factors. Second International Lymphokine Workshop (Ermatingen, Switzerland, May 27-31,1979)
J. Immunol. 123(6):2928-2929

Axelsson, C. and Jarnum, S. (1977) Assessment of the therapeutic value of an elemental diet in chronic inflammatory bowel disease.
Scand. J. Gastroenterol. 12:89-95

Bach, F.H. Alter, B.J., Solliday, S., Zoschke, D.C. and Janis, M. (1970) Lymphocyte reactivity in vitro. II. Soluble reconstituting factor permitting response of purified lymphocyte.
Cell Immunol. 1:219-27

Baker, P.E., Gillis, S. and Smith, K.A. (1979) Monoclonal cytolytic T-cell lines.
J. Exp. Med. 149:273-8

Baklein, K. and Brandtzaeg, P. (1975) Comparative mapping of the distribution of immunoglobulin-containing cells in ulcerative colitis and Crohn's disease of the colon.
Clin. Exp. Immunol. 22:197-209

Baklien, K. and Brandtzaeg,P. (1976) Immunohistochemical characterisation of local immunoglobulin formation in Crohn's disease of the ileum.
Scand. J. Gastroenterol. 11:447-457.

Balkwill, F. R. and Burke, F. (1989) The Cytokine network.
Immunology Today 10:299-304

Barnes, B.M.R., Allan, S., Taylor-Robinson, C.H., Finn, R and Johnson, P.M. (1990) Serum antibodies reactive with Saccharamyces Cerevisiae in inflammatory bowel disease: is IgA antibody a marker for Crohn's disease.
Int. Arch. Allergy Appl. Immunol. 92:9-15

Bartholomeusz, F. D. and Shearman, D. J. (1989) Measurement of activity in Crohn's disease.
J. Gastroenterol. Hepatol. 4:81-94

Barton, J. R., Riad, M. A., Moran, A. G. D. and Ferguson, A. (1990) Mucosal immunodeficiency in smokers is reversible and dose dependant. In MacDonald, T.T., Challacombe, S.J., Bland, P.W., Stokes, C. R., Heatley, R. V. and Mowat, A. McI. eds. Advances in Mucosal Immunology. Lancaster: Kluwer Academic Publishers, 9190:504-505

Becker, J.M. and Raymond, J.L. (1986) Ileal Pouchitis and anastomosis.
Ann. Surg. 204:375-381

Bello-Fernandez, C., Oblakowski, P., Meager, A., Duncombe, A. S., Rill, D. M., Hoffbrand, A. V. and Brenner, M. K. (1991) IL-4 acts as a homeostatic regulator of IL-2-induced TNF and IFN-gamma.
Immunology 72:161-6

Bendtzen, K. (1988) Interleukin 1, interleukin 6 and tumor necrosis factor in infection, inflammation and immunity.
Immunol. Lett. 19(3):183-91

Bendtzen, K., Svenson, M., Fomsgaard, A. and Poulsen, L.K. (1989) Native inhibitors (autoantibodies) of IL-1α and TNF.
Immunology Today 10:222

Bergstrand, O. and Hellers. G. (1983) Breast-feeding during infancy in patients who later develop Crohn's disease.
Scand. J. Gastroenterol. 18:903-906

Best, W. R., Becktel, J. M., Singleton, J. W. and Kern, F.(1976) Development of a Crohn's disease activity index: National Cooperative Crohn's Disease Study.
Gastroenterology 70:439-444.

Beutler, B. and Cerami, A.(1986) Cachectin and tumour necrosis factor as two sides of the same biological coin.
Nature 320:584-8

Beutler, B. and Cerami, A. (1988) The common mediator of shock, cachexia and tumour necrosis.
Adv. Immunol. 42:213-31

Beutler, B. D., Greenwald, D., Hulmes, J. D., Chang, M., Pan, Y.C., Mathison, J., Ulevitch, R. and Cerami, A. (1985a) Identity of tumour necrosis factor and the macrophage-secreted factor cachectin.
Nature 316:552-4

Beutler, B., Mahoney, J., Le Trange, N., Pekala, P. and Cerami, A. (1985b) Purification of cachectin, a lipoprotein lipase suppressing hormone secreted by endotoxin-induced RAW 264.7 cells.
J. Exp. Med. 161:984-95

Biemond, I., Burnham, W.R., D'Amaro, J. and Langman, M.J.S. (1986) HLA-A and HLA-B antigens in inflammatory bowel disease.
Gut 27:934-941

Boackle, R.J., Pruitt, K.M. and Mestecky, J. (1974) The interaction of human complement with artificially aggregated preparations of human secretory IgA.
Immunochemistry 11:543-548.

Boirivant, M., Pallone, F., Ciaco, A., Leoni, M., Fais, S. and Torsoli A.(1991) Usefulness of faecal $Alpha_1$-antitrypsin clearance and faecal concentration as early indicator of postoperative asymptomatic recurrence in Crohn's Disease.
Dig. Dis. Sci. 36:347-352.

Bookman, M. A. and Bull, D.M. (1979) Characteristics of isolated intestinal mucosal lymphoid cells in inflammatory bowel disease.
Gastroenterology 77:503

Braegger, C. P., Nicholls, S., Murch, S. H., Stephens, S. and MacDonald, T. T. (1992) Tumour necrosis factor alpha in stool as a marker of intestinal inflammation.
Lancet 339: 89-91

Brandtzaeg, P. (1976) Complex formation between secretory component and human immunoglobulins related to their content of J chain.
Scand. J. Immunol. 5: 411-419

Brandtzaeg, P. (1981) Transport models for secretory IgA and IgM.
Clin. Exp. Immunol. 44:221-32

Brandtzaeg, P.(1983) Immunohistochemical characterisation of intracellular J-chain and binding site for secretory component (SC) in human immunoglobulin (Ig)-producing cells.
Mol. Immunol. 20:941-66

Brandtzaeg, P. and Korsrud, F. R. (1984) Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue.
Clin. Exp. Immunol. 58:709-18

Brandtzaeg, P., Bjerke, K., Kett, K., Kvale, D., Rognum, T.O., Scott, H., Sollid, L.M. and Valnes, K. (1987) Production and secretion of immunoglobulins in the gastrointestinal tract.
Ann. Allergy 59:21-39

Broberger, O. and Perlmann, P. (1959) Autoantibodies in human ulcerative colitis.
J. Exp. Med. 110:657-673

Broberger, O. and Perlmann, P. (1962) Demonstration of an epithelial antigen in colon by means of fluorescent antibodies from children with ulcerative colitis.
J. Exp. Med. 115:13-26

Brydon W. G., Choudari C. P. and Ferguson, A. Immunoglobulin G, Albumin and alpha-1 antitrypsin in gut lavage fluid; specificity for inflammatory bowel disease (submitted to Gut)

Bruce, M.G. and Ferguson A. (1986) The influence of intestinal processing on the immunogenecity and molecular size of absorbed, circulating ovalbumin in mice.
Immunology 59:295-300

Brynskov, J., Freund, I., Ramussen S.N., Lauristen, K., de Muckadell, O. S., Williams, N., MacDonald, A. S., Tanton, R., Molina, F. and Campanin, M. C. (1989) A placebo controlled, double-blind, randomised trial of cyclosporine therapy in active chronic Crohn's disease.
N. Engl. J. Med. 321:845-850

Brynskov, J. and Tvede, N. (1990) Plasma IL-2R and soluble/shed Interleukin-2 receptor in serum of patients with Crohn's disease. Effect of Cyclosporin.
Gut 31:795-799

Burke, D. A. and Axon, A.X.R. (1988) Adhesive Escherichia Coli in inflammatory bowel disease and infective diarrhoea.
Br. J. Med. 297:102-103

Burke, D.A., Axon, A.T.R., Clayden, S.A., Dixon, M.F., Johnston, D. and Lacey, R.W. (1990) The efficacy of Tobramycin in the treatment of ulcerative colitis.
Aliment. Pharmacol. Ther. 4:123-129

Burnham, W.R. and Lennard-Jones, J.E. (1978) Mycobacteria as a possible cause of inflammatory bowel disease.
Lancet ii:693-696

Camilleri, M and Proano, M. (1989) Advances in the assessment of disease activity in inflammatory bowel disease.
Mayo Clin. Proc. 64:800-807.

Campbell, I.L., Iscaro, A. and Harrison, L.C. (1988) IFN-gamma and tumour necrosis factor-alpha cytotoxicity to murine islet of Langerhans.
J. Immunol. 141:2325-9

Crama-Bohbouth, G. E., Arndt, J. W., Pena, A. S., Verspaget, H. W., Tjon A Tham, R. T., Weterman, I. T., Pauwels, E. K. and Lamers, C. B. (1988) Value of indium-111 granulocyte scintigraphy in the assessment of Crohn's disease of the small intestine: prospective investigation.
Digestion 40:227-236.

Cantrell,D.A. and Smith, K.A. (1983) Transient expression of interleukin 2 receptors. Consequences for T cell growth.
J. Exp. Med. 158:1895-911

Cantrell, D.A. and Smith, K.A. (1984) The interleukin-2 T cell system: A new cell growth model.
Science 224:1312-1316

Caput, D., Beutler, B., Hartog, K., Thayer, R., Brown-Shimer, S. and Cerami, A. (1986) Identification of a common nucleotide sequence in the 3'-untranslated region of mRNA molecules specifying inflammatory mediators.
Proc. Natl. Acad. Sci. U.S.A 83:1670-4

Carlsson, H.E., Lagercrantz, R. and Perlmann P. (1977) Immunological studies in ulcerative colitis. VIII. Antibodies to colon antigen in patients with ulcerative colitis, Crohn's disease, and other diseases.
Scand. J. Gastroenterol. 12:707-714

Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N. and Williamson, B.(1975) An endotoxin-induced serum factor that causes necrosis of tumors.
Proc. Natl. Acad. Sci. U.S.A. 72:3666-70

Catchpole, H.R. (1950) Serum and tissue glycoproteins in mice bearing transplantable tumours.
Proc. Soc. Exp. Med. 14:118

Chadwick, V. S., Mellor, D. M., Myers, D. B., Selden, A. C., Keshavarzian, A., Broom, M. F. and Hobson, C. H. (1988) Production of peptides inducing chemotaxis and lysosomal enzyme release in human neutrophils by intestinal bacteria in vitro and in vivo.
Scand. J. Gastroenterol. 23:121-8

Chensue S.W., Otterness, I.G., Higashi, G.I., Forsch, C.S. and Kunkel, S.L. (1989) Monokine production by hypersensitivity (Schistosoma mansoni egg) and foreign body (Sephadex bead)- type granuloma macrophages: evidence for sequential production of IL-1 and tumour necrosis factor.
J. Immunol. 142:1281-86

Chinosone, D.C., Simon, P.L. and Smith, P.L. (1990) Interleukin-1: effects on rabbit ileal mucosal ion transport in vitro.
Eur. J. Pharmacol. 180: 217-228

Chiodin, R.J., Van Kruiningen, H.J., Thayer, W.R. Merkal, R.S. and Coutu, J.A. (1984) The possible role of mycobacteria in inflammatory bowel disease.
Dig. Dis. Sci. 29:1073-1079

Chiodin, R.J. (1989) Crohn's disease and the Mycobacterioses: a review and comparison of two disease entities.
Clin. Microbiol. Rev. 2:90-117

Choy, M.Y., Walker-Smith, J.A., Williams, C.B. and MacDonald, T.T. (1990) Differential expression of CD25 (interleukin-2R) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative colitis.
Gut 31:1365-1370

Coffman, R.L., Lebman, D.A. and Shrader, B. (1989) Transforming growth factor ß specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes.
J. Exp. Med. 170:1039-1044

Coloe, P., Wilks, C.R., Lightfoot, D. and Tosolin, F.A. (1986) Isolation of M. Paratuberculosis in Crohn's disease.
Aust. Microbiol. 7:188A

Cominelli, F., Nast, C.C. Llerena, R., Dinarello, C.A. and Zipser. R.D. (1990) Interleukin-1 suppresses inflammation in rabbit colitis : mediation by endogenous prostaglandins.
J. Clin. Invest. 85:582-586

Cooke, W.T., Fowler, D.I., Cox, E.V., Gaddie, R. and Meynell, M.J. (1958) The clinical significance of seromucoids in regional ileitis and ulcerative colitis.
Gastroenterology 34:910-919

Cosnes, J. (1988) Crohn's disease and enteral feeding. Comparative nutritional efficacy of elemental and polymeric nutritive mixtures.
Ann. Gastroenterol. Hepatol. (paris) 24:233-8

Cowdrey, J.S. and Johlin, B.J. (1984) Regulation of the primary in vitro response to TNP-polymerized ovalbumin by T suppressor cells induced by ovalbumin feeding.
J. Immunol. 132:2783-9

Crohn, B.B., Ginzberg, L., Oppenheimer G. (1932) Regional enteritis - a pathologic and clinical entity.
J. Am. Med. Assoc.99:1323-1329

Crowthen, J.S., Drasar, B.S., Goddard, P., Hill, M.J. & Johnson, K. (1973) The effect of a chemically defined diet on the faecal flora and faecal steroid concentration.
Gut 14:790-793

Czerkinsky, C.C., Nilsson, L-A, Nygren, H., Ouchterlony, O. and Tarkowski, A (1983). A solid phase enzyme linked immunospot (ELISPOT) assay for enumeration of specific Antibody Secreting Cells.
J. Immunol. Methods 65:109-121.

Czerkinsky C.C. and Svennerholm, A.M. (1983) Ganglioside GM1 Enzyme linked immunospot assay for simple identification of Heat-labile Enterotoxin producing Escherichia coli.
J. Microbiol. 17:965-9.

Czerkinsky, C.C., Nilsson, L. Å., Ouchterlony, ö., Tarkowski, A. and Gretzer, C. (1984) Detection of single antibody-secreting cells generated after in vitro antigen-induced stimulation of human peripheral blood lymphocytes.
Scand. J. Immunol. 19:575-579

Dalziel, T.K. (1913) Chronic interstitial enteritis.
Br. Med. J. 2:1068-1070

Danis, V.A., Harries, A.D. and Heatley, R.V. (1984) Antigen-antibody complexes in inflammatory bowel disease.
Scand. J. Gastroenterol. 19:603-606

Das, P.K., Blaauwgeers, J.L.G., Slob, A.W., Spies, J., Chand, A., Kolk, A. and Houthoff, H.J. (1988) On the mycobacterial aetiology of Crohn's disease: relevant immunological studies.
Gastroenterology 94:A88

Das, K.M., Kadons, Y. and Fleischer, G.M. (1989) Antibody dependent cell mediated cytotoxicity in serum samples from patients with UC.
Am. J. Med.77:791-796

De Dombal, F. T., Burton, I. L., Clamp, S. E. and Goligher, J.C. (1974) Short term course and prognosis of Crohn's disease.
Gut 15:435-443.

Delacroix, D.L., Dive, C., Rambaud, J.C. and Vaerman, J.P. (1982) IgA subclasses in various secretions and in serum.
Immunology 47:383-385

de la Heuerga, J., Dublin, A., Kusher, D. S., Diniewicz, H. A. and Popper, H. (1956). Studies of serum mucoprotein (seromucoid); turbidimetric method.
J. Lab. Clin. Med. 47:403-408

Deem, R.L., Shanahan, F. and Targan, S. R. (1991) Triggered human mucosal T cells release tumour necrosis factor-alpha and interferon-gamma which kill human colonic epithelial cells.
Clin. Exp. Immunol. 83:79-84

Dexter, T.M. and Moore, M. (1986) Growth and development in the haemopoietic system: the role of lymphokines and their potential in disease and malignancy.
Carcinogenesis 7:509-16

DiPalma J.A., Brady, C.E., Stewart, D.L., Karlin, D. A., Mckinney, M. K., Clement, D. J., Coleman, T. W. and Pierson. W. P. (1984) Comparison of colon cleansing methods in preparation for Colonoscopy.
Gastroenterology 86:856-860.

Dinarello, C.A., Cannon, J.G., Wolff, S.M., Bernheim, H.A., Beutler, B., Cerami, A., Figari, I.S., Palladino. M.A. Jr. and O'Connor, J.V. (1986) Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1.
J. Exp. Med. 163:1433-50

Djeu, J.Y., Blanchard, D.K., Halkias, D. and Friedman, H. (1986) Growth inhibition of Candida albicans by human polymorphonuclear neutrophils: activation by interferon-gamma and tumor necrosis factor.
J. Immunol. 137:2980-4

Doe, W.F., Booth, C.C. and Brown, D.L. (1973) Evidence for complement-binding immune complexes in adult celiac disease, Crohn's disease and ulcerative colitis.
Lancet i:402-403

Donnelly, R. P., Fenton, M. J., Finbloom, D. S. and Gerrard, T. L. (1990) Differential product of IL-1 by IFN and IL4.
J. Immunol. 145:569-575.

Durum, S., Quinn, D. G. and Muegge, K. (1991) New cytokines and receptors make their debut in San Antonio.
Immunology Today 12(2):54-7

Drummond, J.C. and Wilbraham, A. (1959) The Englishman's food. London: Jonathan Cape :254

Ebert, E.C. (1990) Intra-epithelial lymphocytes: interferon-gamma production and suppressor/cytotoxic activities.
Clin. Exp. Immunol. 82:81-5

Eddie, D.S., Schulkind, M.L. and Robbins, J.B. (1971) The isolation and bacteriological activities of purified secretory IgA and IgG anti-Salmonella typhimurium 'O' antibodies from rabbit intestinal fluid and colostrum.
J. Immunol. 106:181-190

Edjerham, J., Finkel, Y. and Strandvik, B. (1989) (1989) Na, K-ATPase activity in rectal mucosa of children with ulcerative colitis and Crohn's disease.
Scand. J. Gastroenterol. 24:1121-1125

Elmsgreen, J., Both, H.and Binder, V. (1985) Familial occurrence of complement dysfunction in Crohn's disease: correlation with intestinal symptoms and hypercatabolism of complement.
Gut 26:151-157

Emery, P. and Salmon, M. (1991) Systemic mediators of inflammation.
Br. J. Hosp. Med. 45(3):164-168

Endo, K., Sato, T., Unanoura, A. and Okata, T. and Watanabe, H.(1985). Four cases of ulcerative colitis treated unsuccessfully with an elemental diet. Therapeutic effect of an elemental diet on ulcerative colitis.
Nippon Shokakibyo Gakkai Zasshi 82(2):306-9

Entrican, J.H., Busuttil, A. and Ferguson, A. (1987) Are the focal microscopic jejunal lesions in Crohn's disease produced by a T cell mediated immune response?
Scand. J. Gastroenterol. 22:1071-5

Fais, S., Capobianchi, M.R., Pallone, F., DiMarco, P., Boirivant, M., Dianzani, F. and Torsoli, A. (1991) Spontaneous release of interferon-τ by intestinal lamina propria lymphocytes in Crohn's disease. Kinetics of in vitro response to interferon-τ inducers.
Gut 32:403-407

Fanger, M.W., Shen, L., Pugh, J. and Bernier, G.M. (1980) Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA.
Proc. Natl. Acad. Sci. (USA) 77:3640-3644

Fegan, E. A., Dyck, R. F., Maton, P. N., Hodgson, H. J. F., Chadwick, V. S., Petrie, A., Pepys, M. B. (1982) Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis.
Eur. J. Clin. Invest. 12:351-359.

Fiocchi,C., Hilfiker, M.L., Youngman, K.R., Doerder, C.N. and Finke, J.H. (1984) Interleukin 2 activity of human intestinal mucosa mononuclear cells. Decreased levels in inflammatory bowel disease.
Gut 86:734-42

Flanagan, J.G. and Rabbitts, T.H. (1982) Arrangement of human immunoglobulin heavy-chain constant region genes implies evolutionary duplication of a segment containing τ, ϵ, and α genes.
Nature 300:709-713

Florent, C., L'Hirondel, C., Desmazures, C., Aymes, C. and Bernier, J. J. (1981) Intestinal clearance of $Alpha_1$-antitrypsin: A sensitive method for the detection of protein-losing enteropathy.
Gastroenterology 81:777-780.

Fomsgaard, A., Svenson, M. and Bendtzen, K. (1989) Auto-antibodies to tumour necrosis factor alpha in healthy humans and patients with inflammatory diseases and gram-negative bacterial infections.
Scand. J. Immunol. 30:219-223

Forrest, B.D. (1988) Identification of an intestinal immune response using peripheral blood lymphocytes.
Lancet 332:81-83

Fox, C.C., Lichstein, L.M. and Rocche, J.K. (1987) Immediate hypersensitivity response in idiopathic inflammatory bowel disease (IBD): histamine release from human intestinal mucosal mast cells (MC) in response to gut epithelial antigens.
Gastroenterology 92:1394

Fujii, M., Sugamura, K., Sano, K., Nakai, M., Sugita, K and Hinuma, Y. (1986) High affinity receptor mediated internalisation and degradation of interleukin 2 in human T cells.
J. Exp. Med. 163:550-62

Fujita, K., Naito, S., Okabe, N. and Yao, T. (1984) Immunological studies in Crohn's disease. I. Association with HLA system in the Japanese.
J. Clin. Lab. Immunol. 14:99-102

Fujita, T., Shibuya, H., Ohashi, T., Yamanashi, K. and Tanigichi, T.(1986) Regulation of human interleukin-2 gene: Functional DNA sequences in the 5' flanking region for the gene expression in activated T lymphocytes.
Cell 46:401-407

Fujita, T., Takaoka, C., Matsui, H. and Taniguchi, T. (1983) Structure of the human interleukin 2 gene.
Proc. Natl. Acad. Sci. U.S.A 80: 7437-41

Gaspari, M. M., Brennan, P. T., Soloman, S. M. and Elson C. O. (1988) A method of obtaining, processing, and analysing human intestinal secretions for antibody content.
J. Immunol. Methods 110: 85-91.

Gearhart, P.J. and Cebra, J.J. (1979) Differentiated B lymphocytes: Potential to express particular antibody variable and constant regions depends on site of lymphoid tissue and antigen load.
J. Exp. Med. 149:216-227

Gearhart, P.J., Horwitz, J.L. and Cebra, J.J. (1980) Successive switching of antibody isotype expressed within the lines of a B-cell clone.
Proc. Natl. Acad. Sci. USA 77:5424-5428

Gerry, I. and Walksman, B.H. (1972) Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s).
J. Exp. Med. 136(1):143-55

Giaffer, M.H., North, G. and Holdsworth, C.D. (1990) Controlled trial of polymeric versus elemental diet in the treatment of active Crohn's disease.
Lancet 335:816-819

Gillis, S., Baker, P.E., Ruscetti, F.W. and Smith, K.A. (1978) Long-term culture of human antigen-specific cytotoxic T-cell lines.
J. Exp. Med. 148:1093-8

Gillis, S., Crabtree, G.R. and Smith, K.A. (1979) Glucocorticoid-induced inhibition of T cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation.
J. Immunol. 123:1624-31

Gillis, S. and Smith, K.A. (1977a) In vitro generation of tumor-specific cytotoxic lymphocytes. Secondary allogeneic mixed tumor lymphocyte culture of normal murine spleen cells.
J. Exp. Med. 146(2):468-82

Gillis, S. and Smith, K.A. (1977b) Long term culture of tumour-specific cytotoxic T cells [letter].
Nature 268:154-6

Ginsberg, A.L. and Albert, M.B. (1989) Treatment of patient with severe steroid-dependent Crohn's disease with non-elemental formula diet: Identification of a possible aetiological factor.
Dig.Dis.Sci 34:1624-1628

Gitnick, G., Collins, J., Beaman, B., Brooks, D., Arthur, M., Imaeda, T. and Palieschesky, M. (1989) Preliminary report on the isolation of Mycobacteria from patients with Crohn's disease.
Dig. Dis. Sci. 34:925-932

Graham, D.Y., Markesich, D.C. and Yoshimura, H. H. (1987) Mycobacteria and inflammatory bowel disease: results of culture.
Gastroenterology 92:436-442

Granelli-Piperno, A., Inaba, K. and Steinman, R. M. (1984) Stimulation of lymphokine release from T lymphoblasts. Requirement for mRNA synthesis and inhibition by Cyclosporin A.
J. Exp. Med. 160: 1792-1802

Greenberg, G.R., Fleming C.R., Jeejeebhoy, K.N., Rosenberg, I.H., Sales, D. and Treman, W.J. (1988) Controlled trial of bowel rest and nutritional support in the management of Crohn's disease.
Gut 29:1309-1315

Greenspan E.M. (1955) Clinical significance of serum mucoproteins.
Adv. Intern. Med. 7:101-123

Haagsma, J., Mulder, C.J.J., Eger, A., Bruin, J., Van Ketel, R.J. and Tytgat, G.N.J. (1989) Mycobacterium paratuberculosis is isolated from patients with Crohn's disease in the Netherlands.
Scand. J. Gastroenterol. 24:S158:168A

Halstensen, T.S., Mollnes, T.E., Fausa, O. and Brandtzaeg, P. (1989) Deposits of terminal complement complex (TCC) in the muscularis mucosae and submucosal vessels in ulcerative colitis and Crohn's disease of the colon.
Gut 30:361-6

Haneberg, B. (1974) Immunoglobulins in faeces from infants fed human or bovine milk.
Scand. J. Immunol. 3:191-197

Harvey, R. F. and Bradshaw, J.M. (1980) A simple index of Crohn's disease activity.
Lancet 1:514.

Harries, A. D., Fitzsimons, E., Fifield, R., Dew, M. J. and Rhodes J. (1983) Platelet count: a simple measure of activity in Crohn's disease.
Br Med J 286:1476.

Hatakeyama, M., Tsudo, M., Minamoto, S., Kono, T., Doi, T., Miyata, T., Miyasaka, M. and Tanigushi, T. (1989) Interleukin-2 receptor ß chain gene:generation of three receptor forms by cloned human α and ß chain cDNA's.
Science 244:551-556.

Heberman, R., Ortaldo, J. and Bonnard, G. (1979) Augmentation by Interferon of human natural and antibody-dependent cell cytotoxicity.
Nature 277:221-3

Held, W., Stucki, M., Heusser, C. and Blaser, K. (1989) Production of human antibodies to bee venom phospholipase A_2 *in vitro*.
Scand. J. Immunol. 29:203-209

Herzberg, V.L. and Smith, K.A. (1987) T-cell growth without serum.
J. Exp. Med. 139:998-1004

Holbrook, N.J., Smith, K.A., Fornace, A.J. Jr., Comeau, C.M., Wiskocil, R.L. and Crabtree, G.R. (1984) T-cell growth factor: complete nucleotide sequence and organization of the gene in normal and malignant cells.
Proc. Natl. Acad. Sci. U.S.A. 81:1634-8

Holt, P.G., Sedgwick, J.D., O'Leary, C. and Kirska, K. (1984) ELISA Plaque Assay for the detection of antibody secreting cells. Observations on the nature of the solid phase and on the variations of plaque diameter.
J. Immunol. Methods 74:1-7.

Horibe, K. (1987) Studies on the absorption of elemental diets containing "small peptides" as a nitrogen source.
Nippon Geka Gakkai Zasshi 88:808

Husby S., Jensenius, JC. and Svehag, S.É. (1986) Passage of undegraded dietary antigen into the blood of healthy adults. Further characterisation of the kinetics of Uptake and the size distribution of antigen.
Scand. J. Immunol. 24:447-455

Hutchings, P. R., Cambridge, G., Tite, J. P., Meager, T. amd Cooke, A. (1989) The detection of cytokine secreting cells in mice and man and the clinical application of these assays.
J. Immunol. Methods 120:1-8

Hyams, J.S., Treem, W.R., Eddy, E., Wyzga, N. and Moore, R.E. (1991) Tumour necrosis factor-α is not elevated in children with inflammatory bowel disease.
J. Paed. Gastr. Nutr. 12:233-236

Ingraham, R. and Bussard, A.E (1964). Application of a localised haemolysin reactiom for specific detection of individual antibody producing cells.
J. Exp. Med. 119:667-84

Jackson, P.G., Baker, R.W.R., Lessof, M.H., Ferrett, J. and MacDonald, D.M. (1986) Intestinal permeability in patients with eczema and food allergy.
Lancet ii:1285-6

Jalkanein, S., Reichert, R.A., Gallatin, W.M., Bargatze, R.F., Weissman, I.L. and Butcher, E.C. (1986) Homing receptors and the control of lymphocyte migration.
Immunol. Rev. 91: 39-60

James, A.H. (1977) Breakfast and Crohn's disease.
Br. Med. J. i:943-945

James, S.P. (1988) Remission of Crohn's disease after HIV infection.
Gastroenterology 95:1667-9

James, S.P. and Graeff, A.S. (1987) Effect of IL-2 on immunoregulatory function of intestinal lamina propria T cells in normal non-human primates.
Clin. Exp. Immunol. 70:394-402

James, S.P., Kwan, W.C. and Sneller, M.C. (1990) T cells in inductive and effector compartments of the intestinal mucosa immune system of nonhuman primates differ in lymphokine mRNA expression, lymphokine utilisation, and regulatory function.
J. Immunol. 144:1251-1256

Jannemieke, M.T. and Versteegen, J.M.T. (1988) Enumeration of IFN-Gamma producing human lymphocytes by Spot-Elisa. A method to detect lymphokine-producing lymphocytes at the single cell level.
J. Immunol. Methods 111:25-29

Jansen, K. B., Jarnum, S., Koudahl, G. and Kristensen M. (1976) Serum orosomucoid in ulcerative colitis: its relation to clinical activity, protein loss and turnover of albumin and IgG.
Scand. J. Gastroenterol. 11:177-183.

Jerne, N.K. and Nordin, A.A (1963). Plaque formation in agar by Single Antibody-producing Cells.
Science 140:405.

Jewell, D.P., and Truelove S.C. (1972) Circulating milk antibodies to cow's milk proteins in ulcerative colitis.
Gut 13:796-801

Jonard, P.P., Rambaud, J.C., Dive, C., Vaerman, J.P., Galian, A. and Delacroix, D. L. (1984) Secretion of immunoglobulins and plasma proteins from the jejunal mucosa. Transport rate and origin of polymeric IgA.
J. Clin. Invest. 74:525-535

Jones, E.G., Beeken, W.L., Roessner, K.D. and Brown, W.R. (1976) Serum and intestinal fluid immunoglobulins and jejunal IgA secretion in Crohn's disease.
Digestion 14:12-9

Jones, V.A. (1987) Comparison of total parenteral nutrition and elemental diet in the induction of remission of Crohn's disease. Long term maintenance by personalised food exclusion diets.
Dig. Dis. Sci. 32 (122 Suppl):100s-107s.

Ju, G., Collins, L., Kaffka, K.L., Tsien, W.H., Chizzonite, R., Crowl, R., Bhatt, R. and Kilian, P.L. (1987) Structure-function analysis of human interleukin-2. Identification of amino acid residues required for biological activity.
J. Biol. Chem. 262:5723-31

Juhlin, L., Krause, U and Shelley, W.B. (1980) Endotoxin-induced microclots in ulcerative colitis and Crohn's disease.
Scand. J. Gastroenterol. 15: 311-4

Kasakura, S. and Lowenstein, L. (1965) [Haematology]. A factor stimulating DNA synthesis derived from the medium of leucocyte culture.
Nature 5012:794-795

Kantele, A., Arvilommi, H. and Jokinen, I. (1986) Specific immunoglobulin secreting human blood cells after peroral vaccination against salmonella typhi.
J. Infect. Dis. 153:1126-1131

Katz, K.D., Hollander, D., Vadheim, C.M., McElree, C., Delahunty, T., Dadufaiza, V.D., Krugliak, P. and Rotter, J.I. (1989) Intestinal permeability in patients with Crohn's disease and their healthy relatives.
Gastroenterology 97:927-931

Kawanishi, H., Saltzman, L.E. and Strober, W. (1983) Mechanisms regulating IgA class-specific immunoglobulin production in murine gut-associated lymphoid tissue. 1. T-cells derived from peyer's patches that switch sIgM cells to sIgA B cells in vitro.
J. Exp. Med. 157:433-450

Keicho, N., Kitumura, K., Takaku, F. and Yotsumoto, H. (1990) Serum concentration of soluble interleukin-2 receptor as a sensitive parameter of disease activity in sarcoidosis.
Chest 98:1125-9

Kehoe, J.E., Harvey, L.P. and Daly, J.M. (1986) Alteration of chemotherapy toxicity using a chemically defined liquid diet in rats.
Cancer Res. 46:4047-52

Kesha V.S., Lawson, L., Chung, L.P., Stein, M., Perry, V. H. and Gordon, S. (1990) TNF mRNA localised to paneth cells in normal murine intestinal epithelium.
J. Exp. Med. 171:327-332

Kett, K. and Brandtzaeg, P. (1987) Alterations in local IgA subclass distribution in ulcerative colitis and Crohn's disease of the colon.
Gut 28:1013-1021

Kett, K., Brandtzaeg, P and Fousa, O. (1988). J-chain expression is more prominent in IgA2 than IgA1 colonic immunocytes and is decreased in both subclasses associated with inflammatory bowel disease.
Gastroenterology 94:1429-25

Kett, K., Brandtzaeg, P., Radl, J., Haaijman, J. J. (1986) Different subclass distribution of IgA-producing cells in human lymphoid organs and various secretory tissues.
J. Immunol. 136:3631-3635

Kett, K., Rognum, T. O and Brandtzaeg, P. (1987) Mucosal subclass distribution of immunoglobulin G-producing cells is different in ulcerative colitis and Crohn's disease of the colon.
Gastroenterology 93:919-924.

Kim, P. H. and Kagnoff, M. F. (1990a) Transforming growth factor-beta 1 is a co-stimulant in IgA production.
J. Immunol. 144:3411-3416

Kim, P. H. and Kagnoff, M. F. (1990b) TGF-ß IgA isotype switching at the clonal level.
J. Immunol. 145:3773-3778

Kindler, V., Sappino, A-P., Grau, G.E., Piguet, P-F. and Vassali, P. (1989) The inducing role of tumour necrosis factor in the development of bacterial granulomas during BCG infection.
Cell 56:731-740

Kirsner, J. B. and Spencer, J.A. (1963) Familial occurrence of ulcerative colitis, regional enteritis and ileocolitis.
Ann. Intern. Med. 59:133-144

Klein, J.R. and Kagnoff, M. (1984) Nonspecific recruitment of cytotoxic effector cells in the intestinal mucosa of antigen-primed mice.
J. Exp. Med. 160:1931-6

Klemola, T., Savilahti, E. and Koskimies, S. (1988) Transient IgA and IgM deficiencies are frequent in children with ulcerative colitis.
Eur. J. Pediatrics 147:184-187

Knoflach, P., Park, B.H., Cunningham, R., Weiser, M.M., Albini, B. (1987) Serum antibodies to cow's milk proteins in ulcerative colitis and Crohn's disease.
Gastroenterology 92:479-485.

Kobayashi, K., Allred, C., Cohen, S. and Yoshida, T. (1985) Role of interleukin 1 in experimental pulmonary granuloma in mice.
J. Immunol. 134:358-64

Koninckx, C.R., Giliams, J.P., Polanco, I., and Peña, A.S. (1984) IgA antigliadin antibodies in coeliac and inflammatory bowel disease.
J. Paed. Gastroenterol. Nutr. 3(5):676-682

Korsmeyer, S.J., Williams, Jr. R.C., Wilson, I.D., and Strickland, R.G. (1975) Lymphocytic antibodies in inflammatory bowel disease. A family study.
N. Engl. J. Med. 293:1117-1120

Koukkou, E., Panayiotidis, P., Alevizou-Terzaki, V. and Thalassinos, N. (1991) High levels of serum soluble interleukin-2 receptors in hyperthyroid patients. Correlation with serum thyroid hormones and independence from the aetiology of the hyperthyroidism.
J. Clin. Endocrinol. Metab. 73:771-6

Kutteh, W.B., Koopman, W. H., Conley, M. E., Egan, M. L. and Mestecky, J. (1980) Production of predominantly polymeric IgA by human peripheral blood. Lymphocytes stimulated in vitro with mitogens.
J. Exp. Med. 152 :1424-1429

Kutteh, W.H., Prince, S.J. and Mestecky, J. (1982) Tissue origins of human polymeric and monomeric IgA.
J. Immunol. 128:990-995

Kruis, W., Paulus, W., Fateh-Moghadam, A., Schüssler, P. and Eisenburgh, J. (1981) Serum immunoglobulin concentrations in Crohn's disease. Clinical relevance and comparison with lipid-A-antibody titers.
Z. Gastroenterol. 19:276-283

Lebman, D.A., Lee, F.D. and Coffman, R.L. (1990) Mechanism for transforming growth factor ß and IL-2 enhancement of IgA expression in lipopolysaccharides-stimulated B-cell cultures.
J. Immunol. 144:942-959

Lee, F. K., Nahmias, A. J., Lowery, S., Nesheim, S., Reef, S., Thompson, S., Oleske, J., Vahlne, A. and Czerkinsiky, C. (1989) ELISPOT: A new approach to studying the dynamics of virus-immune system interaction for the diagnosis and monitoring of HIV infection.
Aids Research and Human Retroviruses 5(5):517-523

Le Quintrec, Y., Cosnes, J., Le Quintrec, M., Contour, J.F., Baumer, P., Bellanger, J., and Gendre, J.P. (1987) Exclusive elemental diets in cortico-resistant and cortico-dependent forms of Crohn's disease.
Gastroenterol. Clin. Biol. 11(6-7):477-82.

Lerner, A., Rossi, T.M., Park, A., Albini, B., Lebenthal, E. (1989) Serum antibodies to cow's milk proteins in paediatric Inflammatory bowel disease.
Acta paediatr. Scand. 78:384-389

Lett-Brown, M.A., Alam, R. and Grant, J.A. (1988) Regulation of human basophils and mast cells. Activation by cytokines. In Cruse, J.M. and Lewis, R.E. Jr (eds), The Year in Immunology, 1988. Immunoregulatory cytokines and cell growth, pp 195-204 (Basel:Karger)

Lewkonia, R.M. and McConnel, R.B. (1976). Familial inflammatory bowel disease - hereditary or environment?
Gut 17:235-41

Liew, F.Y., Li, Y. and Millot, S. (1990) Tumour necrosis factor (TNF-alpha) in leishmaniasis. II. TNF-alpha induced macrophage leishmanicidal activity is mediated by nitric oxide from L-arginine.
Immunology 71:556-9

Ligumsky, M., Simon, P.L., Karmeli, F. and Rachmilewitz, D. (1990) Role of IL-1 in IBD: enhancing duration of active disease.
Gut 31:686-689

Lindh, E. (1975) Increased resistance of IgA to proteolytic degradation after binding of secretory component.
J. Immunol. 114:284-286

Lockey, E., Anderson, A.J. and Maclagan, N.F. (1956) Urine and Serum mucoproteins in cancer and other diseases.
Brit. J. Cancer 10:209

Logan, R.F.A., Gillon, J., Ferrington, C. and Ferguson, A. (1981) Reduction of gastrointestinal protein loss by elemental diet in Crohn's disease of the small bowel.
Gut 22:283-287

Loughnan, M. S. and Nossal, G. J. V. (1989) Interleukin-4 and 5 control expression of IL-2 receptor on murine B cells through independent induction of its two chains.
Nature 340:76-79

Loughnan, M. S., Takatsa, K., Harada, N. and Nossal, G.J. (1987) T-cell replacing factor (interleukin 5) induces expression of interleukin 2 receptors on murine splenic B cells.
Proc. Natl. Acad. Sci. U.S.A. 84(15):5399-5403

Lorusso, D., Misciagna and Guerra, V. (1989) Cigarette smoking and ulcerative colitis a case control study.
Hepatogastroenterology 36:202-204

Lowenthal, J.W. and Green, W.C. (1987) Contrasting interleukin-2 binding properties of the α (p55) and ß (p70) protein subunits of the human high affinity interleukin-2 receptor.
J. Exp. Med. 166:1156-1167

Lubega, J. and Jeffrey-Davies, T. (1990) A comparison of serum mucoprotein with serum α-1 acid glycoprotein, haptoglobin, and α-1 antitrypsin assays in monitoring inflammatory bowel disease.
Clin. Chim. Acta. 188:59-70

Lycke, N.R. and Kilander, A., Nilsson, L.A., Tarkowski, A. and Werner, N. (1989) Production of antibodies to gliadin in intestinal mucosa of patients with coeliac disease: A study at the single cell level.
Gut 30:72-77

MacDermott, R.P., Nash, G.S., Bertovich, M.J. Seiden, M.V., Bragdon, M. J., and Beale, M.G. (1981) Alterations in IgM, IgG and IgA synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease.
Gastroenterology 81:844-52

MacDermott, R.P., Nash, G.S., Bertovich, M.J., Mohrmann, R. F., Kodner, I. J., Delacroix, D. L. and Vaerman, J. P. (1986) Altered patterns of secretion of monomeric IgA and IgA subclass 1 by intestinal mononuclear cells in inflammatory bowel disease.
Gastroenterology 91:379-85

MacDermott, R.P. and Stenson, W.F. (1988) Alterations of the immune system in ulcerative colitis and Crohn's disease.
Adv. Immunol. 42:285-328

MacDonald, T.T., Hutchings, P., Choy, M.Y., Murch, S. and Cooke, A. (1990) Tumour necrosis factor-alpha and interferon-gamma production measured at the single cell level in normal and inflamed human intestine.
Clin. Exp. Immunol. 81:301-305

MacDonald, T.T. and Spencer, J. (1988) Evidence that activated mucosal T cells play a role in the pathogenesis of enteropathy in human small intestine.
J. Exp. Med. 167:1341

Maclean, L.D. and Gordon, J. (1965) [Haematology]. A lymphocyte-stimulating factor produced in vitro.
Nature 5012:795-796

Mahida, Y.R., Wu, K., Patel, S. and Jewell, D.P. (1988) Interleukin-2 receptor expression by macrophages in inflammatory bowel disease.
Clin. Exp. Immunol. 74: 382-386

Mahida, Y.R., Gallagher, A., Kurlak, L. and Hawkey, C.J. (1990a) Plasma and tissue interleukin-2 receptor in inflammatory bowel disease.
Clin. Exp. Immunol. 82:75-80

Malchow, H., Steinhardt, H.J., Lorenz-Meyer, H., Strohm, W.D., Rasmussen, S., Sommer, H., Jarnum, S., Brandes, J.W., Leonhardt, H., Ewe, K. and Jesdinsky H. (1990) Feasibility and effectiveness of a defined-formula diet regimen in treating active Crohn's disease. European Co-operative Crohn's Disease Study III.
Scand. J. Gastroenterol. 25:235-244

Marteau, P., Colombel, J.F., Nemeth, J., Vaerman, J.P., Dive, J.C. and Rambaud, J.C. (1990) Immunological study of histologically non-involved jejunum during Crohn's disease: evidence for reduced in vivo secretion of secretory IgA.
Clin. Exp. Immunol. 80:196-201

Matthews, N., Mayberry, J.F., Rhodes, J., Neale, L., Munro, J., Wensinck, F., Lawson, G.H., Rowland, A.C., Berkhoff, G.A. and Barthold, S.W. (1980) Agglutinins to bacteria in Crohn's disease.
Gut 21:376-80

Mayberry, J.F., Rhodes, J. and Newcombe, R.G. (1978) Breakfast and dietary aspects of Crohn's disease.
Br. Med. J. ii:1401

Mayer, L.(1990) Immunology of inflammatory bowel disease. Current Opinion in Gastroenterology 6:556-560

Mayer, L., Eisehardt, D., Salomon, P., Bauer, W., Plous, R. and Piccinini, L. (1991) Expression of class II molecules on intestinal epithelial cells in humans: Differences between normal and inflammatory bowel disease.
Gastroenterology 100:3-12

Mazzei, G. J., Bernasconi, L. M., Lewis, C., Mermod, J. J., Kinder, V. and Shaw, A. R. (1990) Human granulocyte-macrophage colony-stimulating factor plus phorbol myristate acetate stimulate a promyelocytic cell line to produce an IL-1 inhibitor.
J. Immunol. 145:585-591

Menge, H., Simes, G., Wagner, J., Hahn, H. and Rieken, E.O. (1985) Bacterial colonisation of the rat jejunum in long-term nutrition with an elemental diet.
Klin. Wochenschr. 63(17):827-32

Mestecky, J., Kutteh, W.H., Brown, T.A., Russell, M.W., Phillips, J. O., Moldoveau, Z., Moro, I. and Crago, S. S. (1983) Function and biosynthesis of polymeric IgA.
Annal. N. Y. Acad. Sci. 409:292-306

Metlay, J.P., Pure, E. and Steinman, R.M. (1989) Control of immune responses at the level of antigen-presenting cells: a comparison of the function of dendritic cells and lymphocytes.
Adv. Immunol. 47:5-116

Meuer, S.C., Hussey, R.E., Cantrell, D.A., Hodgdon, J.C., Schlossman, S.F., Smith, K.A. and Reinherz, E. (1984) Triggering of the T3-Ti antigen-receptor complex results in clonal T-cell proliferation through an interleukin 2-dependent autocrine pathway.
Proc. Natl. Acad. Sci. U.S.A 1984: 81(5): 1509-13

Meyers, S., Wolke, A., Field, S. P., Feuer, E. J., Johnson, J. W. and Janowitz, H. D. (1985) Faecal $alpha_1$-antitrypsin measurement: an indicator of Crohn's disease activity.
Gastroenterology 89:13-18

Michie, H.R., Sherman, M.L., Spriggs, D.R., Rounds, J., Christie, M. and Wilmore, D.W. (1989) Chronic TNF infusion causes anorexia but not accelerated nitrogen loss.
Ann. Surg. 209:19-24

Monk, M., Mendeloff, A.I., Siezel, C.I. and Lilienfield, A. (1969) An epidemiological study of ulcerative colitis and regional enteritis among adults in Baltimore. II. Social and demographic factors.
Gastroenterology 56:847-857

Morgan, D.A., Ruscetti, F.W. and Gallo, R. (1976) Selective in vitro growth of T lymphocytes from normal human bone marrows.
Science 193:1007-8

Mostov, K. E. and Blobel, G. (1982) A transmembrane precursor of secretory component. The receptor for transcellular transport of polymeric immunoglobulins.
J. Biol. Chem. 157:11816-21

Mowat, A. McI. (1986) Depletion of suppressor T cells by 2'-deoxyguanosine abrogates tolerance in mice fed ovalbumin and permits the induction of intestinal delayed-type hypersensitivity.
Immunology 58:179-84

Mowat, A. McI. (1987) The regulation of immune responses to dietary protein antigens.
Immunology Today 8:93-98

Mowat, A.McI., Strobel, S., Drummond. H.E. and Ferguson, A. (1982) Immunological responses to fed protein antigens in mice. I. Reversal of oral tolerance to ovalbumin by cyclophosphamide.
Immunology 45(1):105-13

Mueller, Ch., Knoflach, P. and Zielinski, C.C. (1990) T-Cell activation in Crohn's disease: Increased levels of soluble Interleukin-2 receptor in serum and supernatants of stimulated peripheral blood mononuclear cells.
Gastroenterology 98:639-646

Mullin, G.E., Harris, M.L., Bayless, T.M. and James, S.P. (1991) Increased IL-2mRNA in the intestinal mucosal lesions of Crohn's disease but not ulcerative colitis.
Gastroenterology 100(5):A602

Muraguchi, A., Kehrl, J.H., Longo, D.L., Volkman, D.J., Smith, K.A. and Fauci, A.S. (1985) Interleukin 2 receptors on human B cells. Implications for the role of interleukin 2 in human B cell function.
J. Exp. Med. 161:181-97

Murch, S.H., Lamkin, V.A., Savage, M.O., Walker-Smith, J. A. and MacDonald, T.T. (1991) Tumour necrosis factor alpha production in childhood chronic inflammatory bowel disease.
Gut 32: 913-917

Murray, H.W., Stern, J.J. Welte, K., Rubin, B.Y., Carriero, S.M. and Nathan, C.F. (1987) Experimental visceral leishmaniasis: production of interlekin 2 and interferon-ɾ tissue immune reaction and response to treatment with interleukin 2 and interferon-gamma.
J. Immunol. 138:2290-97

Myren, J., Bouchier, I. A. D., Watkinson, G., Softley, A., Clamp, S. E., De Dombal, F. T. (1984) The OMGE multinational inflammatory bowel disease survey 1976-1982: a further report on 2,657 cases.
Scand. J. Gastroenterol. Suppl. 95:1-27.

Nakanishi, K., Malek, T.R., Smith, K.A., Hamaoka, T., Shevach, E.M. and Paul, W.E. (1984) Both interleukin 2 and a second T cell-derived factor in EL-4 supernatant have activity as differentiation factors in IgM synthesis.
J. Exp. Med. 160:1605-21

Nawroth, P.P. and Stern, D.M. (1986) Modulation of endothelial cell hemostatic properties by tumor necrosis factor.
J. Exp. Med. 163:740-5

Neguchi, M., Sasaki, T., Hiwatashi, N. and Toyota, T. (1991) Production of Tumour necrosis factor alpha from the colonic mucosa in inflammatory bowel disease.
Gastroenterology 100:A604

Nicklin, S. (1987) Intestinal uptake of antigen - immunological consequences. In Miller, I.K. and Nicklin, S. (eds), Immunology of the Gastrointestinal Tract, pp. 87-109 (Boca Raton, FL: CRC Press)

Nowell, P.C. (1960) Phytohaemagglutinin: An initiator of mitosis in cultures of normal human leukocytes.
Cancer Research 20:462-466

Okada, M., Yao, T., Yamamoto, T., Takenaka, K., Imamura, K., Maeda, K. and Fujita, K. (1990) Controlled trial comparing an elemental diet with prednisolone in the treatment of Crohn's disease.
Hepatogastroenterology 37(1):72-80

O'keefe, S.J., Ogden, J., Rund, J. and Potter, P. (1989) Steroids and bowel rest versus elemental diet in the treatment of patients with Crohn's disease:the effects on protein metabolism and immune function.
J. Parenter. Enteral. Nutr. 13:455-60

Old, L.J. (1985) Tumour necrosis factor (TNF).

Oliff, A., Defeo-Jones, D., Boyer, M, Martinez, D., Kiefer, D., Vuocolo, G., Wolfe, A. and Socher, S.H. (1987) Tumours secreting human TNF/Cachectin induce cachexia in mice.
Cell 50:555-563.

O'Mahony, S., Arranz, E., Barton, J. R. and Ferguson, A. (1991a) Dissociation between systemic and mucosal humoral immune responses in coeliac disease.
Gut 32:29-35

O'Mahony, S., Barton, J. R., Crichton, S., Ferguson, A. (1990) Appraisal of gut lavage in the study of intestinal humoral immunity.
Gut 31:1341-1344.

O'Mahony, S., Choudari, C. P., Barton, J. R., Walker, S. and Ferguson, A. (1991b) Gut lavage fluid proteins as markers of activity in inflammatory bowel disease.
Scand. J. Gastroenterol. 26:940-944.

O'Morain, C.A., Abelow, A.C., Chervu, L.R., Fleichner, G.M. and Das, K.M. (1986) Chromium 51-ethylene diamine tetraacetate test: a useful test in the assessment of inflammatory bowel disease.
J. Lab. Clin. Med. 108(5):430-5

O'Morain, C., Segal, A.W.and Levi, A.J. (1980) Elemental diets in the treatment of acute Crohn's disease.
Br. Med. J. 281:1173-5

O'Morain, C., Segal, A, W. and Levi, A.J.(1984) Elemental diet in the treatment of acute Crohn's disease: a controlled trial.
Br. Med. J. 288: 1859-1862

Onderdonk, A.B., Bronson, R. and Cisneros, R. (1987) Comparison of Bacteroides vulgatus strains in the enhancement of experimental ulcerative colitis.
Infect. Immunol. 55:835-836

Park, R.H.R., Galloway, A., Danesh, B.J.Z. and Russell, R.I. (1991) Double-blind controlled trial of elemental and polymeric diets as primary therapy in active Crohn's disease.
Eur. J. Gastroenterol. Hepatol. 3:483-490

Park, R.H.R., Khasawneh, S., Duncan, A., Russell, R.I., Hutchison, A. and Shenkin, A. (1988) Acute phase response in malnourished patients with Crohn's disease.
Clin. Nutr. 7: 207-211.

Peters, T., Jr (1980) Proteins. In the Chemical Diagnosis of disease, 2nd edition. (Eds. Brown. S.S., Mitchell, F.L. and Young, D.S.) Elsevier North Holland.

Phillips, J. O., Everson, M.P., Moldoveanu, Z., Lue, C. and Mestecky, C. (1990) Synergistic effect of IL-4 and IFN-τ on the expression of polymeric Ig receptor (secretory component) and IgA binding by human epithelial cells.
J. Immunol. 145:1740-1744

Plaut, A.G. (1983) The IgA1 proteases of pathogenic bacteria.
Ann. Rev. Microbiol. 37: 603

Poo, W.J., Conrad, L. and Janeway, C.A. Jr. (1988) Receptor-directed focusing of lymphokine release by helper T cells.
Nature 332:378-80

Porat, O. (1989) The effect of tumour necrosis factor alpha on the activity of lipoprotein lipase in adipose tissue.
Lymphokine Res. 8(4):459-469

Potter, B, J., Hodgson, H.J.F., Mee, A.S. and Jewell, D.P. (1979) C1q metabolism in ulcerative colitis and Crohn's disease.
Gut 20:1012-1019

Powell-Tuck, J., Bown, R. L. and Lennard-Jones, J. E. (1978) A comparison of oral prednisolone given as a single or multiple daily doses for active proctocolitis.
Scand. J. Gastroenterol. 13:833-837.

Raouf, A.H., Hildrey, V., Daniel, J., Walker, R.J., Elias, E. and Rhodes, J.M. (1991) Enteral feeding as sole treatment for Crohn's disease: controlled trial of whole protein vs amino acid based feed and a case study of dietary change.
Gut 32:702-707.

Rawcliffe, P.M. and Truelove, S.C. (1978) Breakfast and Crohn's disease. 1.
Br. Med. J. ii:539-540

Richens, E.R., Thorp, C.M., Bland, P.W. and Hall, N.D. (1982) Circulating immune complexes in Crohn's disease. Their characterisation and interrelationship with components of the complement system.
Dig. Dis. Sci. 27:129-138

Richman, L.K. Chiller, J.M., Brown, W.R. Hanson, D.G. and Vaz, N.M. (1978) Enterically induced immunologic tolerance. I. Induction of suppressor T lymphoyctes by intragastric administration of soluble proteins.
J. Immunol. 121(6):2429-34

Robb, R. J., Green, W. C. and Rusk, C. M. (1984) Low and high affinity cellular receptors for interleukin 2. Implications for the level of Tac antigen.
J. Exp. Med. 160(4):1126-46

Robb, R.J. and Smith, K.A. (1981) Heterogeneity of human T-cell growth factor(s) due to variable glycosylation.
Mol. Immunol. 18(12):1087-94

Robb, R.J. and Warner, C.G. (1983) Direct demonstration of the identity of T cell growth factor binding protein and the TAC antigen.
J. Exp. Med. 158:1332

Robertson, D.A.F., Ray, J., Diamond, I. and Edwards, J.G. (1989) Personality profile and affective state of patients with inflammatory bowel disease.
Gut 30:623-626

Rognum, T.O., Brandtzaeg, P., Elgjo, K. and Fausa, O. (1987) Heterogeneous epithelial expression of class II (HLA-DR) determinants and secretory component is related to dysplasia in ulcerative colitis.
Br. J. Cancer 56:419-429

Rouzer, C,A.and Cerami, A. (1980) Hypertriglyceridemia associated with Trypanosoma brucei brucei infection in rabbits: role of defective triglyceride removal.
Mol. Biochem. Parasitol. 2(1):31-8

Rowe, A.H., Rowe, A. and Uyeyama, K. (1953) Regional enteritis - its allergic aspects.
Gastroenterology 23:554-569

Rozen, O., Zonia, J., Yekutiel, P. and Gilat, T. (1979) Crohn's disease in the Jewish population of Tel-Aviv-Yafo.
Gastroenterology 76:25-30

Rubin, L.A., Jay, G. and Nelson, D.L. (1986) The released interleukin 2 receptor binds interleukin 2 efficiently.
J. Immunol. 137:3841-4

Ruseler-van Embden, J.G., van der Helm, R and van Lieshout, L.M. (1989) Degradation of intestinal glycoproteins by Bacteroides vulgatus.
FEMS Microbiol. Lett. 49(1):37-41

Rutgeerts, P., Vantrappen, G., Van Isveldt, J and Geboes, K. (1988) Rifabutin therapy in patients with recurrent Crohn's disease after ileocolonic resection.
Gastroenterology 94:A391S

Sachar, D. B., Smith, H., Chan, S., Cohen, L. B., Lichtiger, S. and Messer, J. (1986) Erythrocyte sedimentation rate as a measure of clinical activity in inflammatory bowel disease.
J. Clin. Gastroenterol. 8:647-650

Saito, Y., Sabe, H., Suzuki, N., Kondo, S., Ogura, T., Shimizu, A. and Honjo, T. (1988) A large number of the L chain (Tac) enhance the association rate of interleukin 2 to the high affinity site of the interleukin-2 receptor.
J. Exp. Med. 168:1563-72

Sanderson, I.R., Boulton, P., Menzies, I. and Walker-Smith, J.A. (1987) Improvement of abnormal lactulose/rhamnose permeability in active Crohn's disease on the small bowel by an elemental diet.
Gut 28:1073-76

Sandler, R.S., Jordan, M.C. and Kupper, L.L. (1988) Development of a Crohn's index for survey research.
J. Clin. Epidemiol. 41:451-458.

Sartor, R.B. (1989) Importance of intestinal mucosal immunity and luminal bacterial cell wall polymers in the aetiology of inflammatory joint diseases.
Bailliere's Clin. Rheumatol. 3:223-45

Saverymuttu, S. H., Cammileri, M., Rees, H., Lavender, J. P., Hodgson, H.J.F. and Chadwick, V.S. (1986) Indium 111-Granulocyte scanning in the assessment of disease extent and disease activity in inflammatory bowel disease.
Gastroenterology 90:1121-1128.

Saverymuttu, S.H., Hodgson, H.J. & Chadwick, V.S. (1985a) Controlled trial comparing prednisolone with an elemental diet plus non-absorbable antibiotics in active Crohn's disease.
Gut 10:994-998

Saverymuttu, S.H., Peters, A.M., Lavender, H.P., Chadwick, V.S. and Hodgson, H.J. (1985b) *In vivo* assessment of granulocyte migration to diseased bowel in Crohn's disease.
Gut 26:378-383

Saverymuttu, S.H., Chadwick, V.S. and Hodgson, H.J. (1985c) Granulocyte migration in ulcerative colitis.
Eur. J. Clin. Invest. 15:60-63

Schmid, K. (1975) α-1 acid glycoprotein. In the Plasma Proteins, Vol 1, 2nd edn (Ed. F.W. Putnam). Chapter 4. Academic Press, New York.

Schreiber, S., Raedler, A., Baldassano, R.N., Conn, R.P. and MacDermott, P.P. (1991) Increased release of soluble interleukin-2 receptor by colonic lamina propria mononuclear cells in inflammatory bowel disease.
Gastroenterology 100:A614

Schultze, H.E. and Heremans, J.F. (1966), Molecular Biology of Human Proteins, Vol 1, Elsevier, Amsterdam.

Scott, B.B., Goodall, A., Stephenson, P. and Jenkins, D. (1983) Rectal mucosal plasma cells in inflammatory bowel disease.
Gut 24: 519-24

Scott, H., Solheim, B.G., Brandtzaeg, P and Thorsby, E. (1980) HLA-DR-like antigens in the epithelium of the human small intestine.
Scand. J. Immunol. 12:77-82

Seckinger, P., Isaaz, S. and Dayer, J.M. (1989) Purification and biological characterisation of a specific tumour necrosis factor alpha inhibitor.
J. Biol. Chem. 264(40):11966-73

Sedgwick, J.P. and Holt, P.G. (1983) A solid phase immunoenzymatic technique for the enumeration of specific antibody Secreting cells.
J. Immunol. Methods 57:301-309.

Sedgwick, J.P. and Holt, P.G. (1986) The Elisa-plaque assay for the detection and enumeration of antibody secreting cells: An overview.
J. Immunol. Methods 87:37-44.

Shaffer, J.L., Hughes, S.D., Linaker, B.D., Baker, R.D. and Turnberg, L.A. (1984) Controlled trial of rifampicin and ethambutol in Crohn's disease.
Gut 25:203-205

Shanahan, F., Randolph, L., King, R., Oseas, R., Brogan, M., Witkop, C., Rotter, J. and Targan, S. (1988) The Hermansky-Pudak syndrome: an immunological assessment in 15 cases.
Am. J. Med. 85:823-828

Sharon, M., Klauser, R.D., Cullen, B.R., Chizzote, R. and Leonard, W.J. (1986) Novel interleukin-2 receptor subunit detected by cross linking under high affinity conditions.
Science 234:859-63

Sherry, B. and Cerami, A. (1988) Cachectin or Tumour necrosis factor exerts endocrine, paracrine and autocrine control of inflammatory responses.
J. Cell Biol. 107:1269-1237

Shorter, R.G., McGill, D.B. and Bahn, R.C. (1984) Cytotoxicity of mononuclear cells for autologous colonic epithelial cells in colonic diseases.
Gastroenterology 86:13-22

Siegel, J.P. Sharon, M., Smith, P.L. and Leonard, W.J. (1987) The IL-2 receptor ß-chain (p70): Role in mediating signals for LAK, NK, and proliferative activities.
Science 238:75-78

Silk, D.B.A., Fairclough, P.D., Clark, M. L., Hegarty, J. E., Marrs, T. C., Addison, J. M., Burton, D., Clegg, K. M., Matthews, D. M. (1980) Use of a peptide rather than free amino acid nitrogen source in chemically defined 'elemental' diets.
J. Parenter. Enteral. Nutr. 4:548-53

Silverstein, M.D., Lashner, B.A. Hanauer, S.B., Evans, A.A. and Kirsner, J.B. (1989) Cigarette smoking in Crohn's disease.
Am. J. Gastroenterol. 84:31-33

Singleton J. W. (1987) Clinical activity assessment in inflammatory bowel disease.
Dig. Dis. and Sci. 32: (Suppl) 42S-45S.

Sipe, J.D., Vogel, S.N., Douches, S. and Neta, R. (1987) Tumor necrosis factor/cachectin is a less potent inducer of serum amyloid A synthesis than interleukin 1.
Lymphokine Res. 6(2):93-101

Smith, J.L., Arteaga, C. and Heymsfield, S.B. (1982) Increased ureagenesis and impaired nitrogen use during infusion of a synthetic amino acid formula.
N. Engl. J. Med. 306:1013-8

Smith, K. A. and Cantrell, D. A. (1985) Interleukin 2 regulates its own receptors.
Proc. Natl. Acad. Sci. USA 82:864-8

Smith, K.A., Gillis, S., Baker, P.E., McKenzie, D.and Ruscetti, F.W. (1979) T-cell growth factor-mediated T-cell proliferation.
Ann. N. Y. Acad. Sci. 332:423-32

Smolen, J.S., Gangl, A., Polterauer, P., Menzel, E.J. and Mayr W.R. (1982) HLA-A antigens in inflammatory bowel disease.
Gastroenterology 82:34-38

Spalding, D.M. and Griffin, J.A. (1986) Different pathways of pre-B cell lines are induced by dendritic cells and T-cells from different lymphoid tissues.
Cell 44:507-515

Stanford, J.R., Ayers, L.W., Cornhill, J.F., Carey, L.G., Kilman, J.W., Pace, W.G. and Vasko, J.S. (1979) Alterations in gastrointestinal contents induced by elemental diet.
J. Surg. Oncol. 12:165-7

Stanford, J.L., Dourmashkin, R., Mcintyre, G. and Visuvanathan, S. (1988) Do Mycobacteria exist in alternative forms and what part do they play in the aetiology in inflammatory bowel disease. In Inflammatory Bowel Disease: Current Status and Future Approach edited by MacDermott RP. Amsterdam: Elsevier Science Publishing, pp 503-508

Statland, B. E., Winkel, P. and Killingsworth, L. M. (1976) Factors contributing to intra-individual variation of serum constituents: 6. Physiological day-to-day variation in concentrations of 10 specific proteins in sera of healthy subjects.
Clinical Chemistry 22:1635-1638

Steffen, M., Abboud, M., Potter, G.K., Yung, Y.P. and Moore, M.A.S. (1989) Presence of tumour necrosis factor or related factor in human basophil/mast cells.
Immunology 66:445-50

Stephen, R.V. and Randal, H.T. (1969) Use of concentrated, balanced, liquid elemental diet for nutritional management of catabolic states.
Ann. Surg. 170:642-667

Strickland, R.G., Robinson, J.M. and Greenelee, L.S. (1980) Circulating interferon in active inflammatory bowel disease.
Gastroenterology 78:A1271

Swarbrick, E.T., Stokes, C.R. and Soothill, J.F. (1979) absorption of antigens after oral tolerance and the simultaneous induction of specific systemic tolerance.
Gut 20:121-125

Strygler, B., Nicar, M. J., Santangelo, W. C., Porter, J. L. and Fordtran, J. S. (1990) $Alpha_1$-antitrypsin excretion in stool in normal subjects and in patients with gastro-intestinal disorders.
Gastroenterology 99:1380-1387.

Taniguchi, T., Matsui, H., Fujita, T., Takaoka, C., Kashima, N., Yoshimoto, R. and Hamuro, J. (1983) Structure and expression of a cloned cDNA for human interleukin-2.
Nature 302:305-10

Takahashi, F. and Das, K.M. (1985) Isolation and characterisation of colonic autoantigen specifically recognised by colon tissue-bound immunoglobulin G from idiopathic ulcerative colitis.
J. Clin. Invest. 76:311-31

Taylor, K.B. and Truelove, S.C. (1961) Circulating antibodies to milk proteins in ulcerative colitis.
Br. Med. J. 2:924-9

Teahon, K., Bjarnason, I., Pearson, M. and Levi, A.J. (1990) Ten years experience with an elemental diet in the management of Crohn's disease.
Gut 31:1133-1137

Teahon, K., Smethurst, P., Pearson, M., Levi, A. J. and Bjarnasson, I. (1991) The effect of elemental diet on the permeability and inflammation in Crohn's disease.
Gastroenterology 101:84-89

Teisberg, P. and Gjone, E. (1975) Humoral immune system activity in inflammatory bowel disease.
Scand. J. Gastroenterol. 10:545-549

Teshigawara, K., Wang, H-M., Kato, K. and Smith, K.A. (1987) Interleukin-2 high affinity receptor expression requires two distinct binding proteins.
J. Exp. Med. 165:223-38

Thayer, W.R., Contu, J.A., Chiodin, R.J. and Van Kruiningen, H.J. (1988) Use of rifabutin and streptomycin in the therapy of Crohn's disease.In Inflammatory Bowel Disease: Current Status and Future Approach edited by MacDermott RP. Amsterdam: Elsevier Science Publishing, pp 565-568

Thompson, H. L., Burbelo, P.D., Yamada, Y., Kleinmam, H.K. and Metcalfe, D.D. (1989) Mast cells chemotax to laminin with enhancement after IgE-mediated activation.
J. Immunol. 143:4188-92

Thornton, J.R., Emmett, P.M. and Heaton, K.W. (1979). Diet and Crohn's disease: Characteristics of pre-illness diet.
Br. Med. J. ii:762-764

Tomasi, T.B., Tan, E.M., Soloman, A. and Prendergast, R.A. (1965). Characteristics of an immune system common to certain external secretion.
J. Exp. Med. 121:101-124

Torti, F.M., Dieckman, B., Beutler, B., Cerami, A. and Ringold, G.M. (1985) A macrophage factor inhibits adipocyte gene expression: an *in vitro* model of cachexia.
Science 229:867-869

Tracey, K.J., Wei, H.E., Manogue, K.R., Fong, Y., Hesse, D. G., Nguyen, H. T., Kuo, G. C., Beutler, B., Cotran, R. S., Cerami, A.and Lowry, S. F. (1988) Cachectin/tumour necrosis factor induces cachexia, anaemia and inflammation.
J. Exp. Med. 167:1211-1227

Troncone, R. and Ferguson, A. (1988) Gliadin presented via the gut induces oral tolerance in mice.
Clin. Exp. Immunol. 72:284-7

Truelove, S. C.and Witts, L. J. (1955) Cortisone in ulcerative colitis: Final report on a therapeutic trial.
Br. Med. J. 2:1041-1048

Tsudo, M., Kozak, R.W. Goldman, C.K. and Waldmann, T.A. (1986) Demonstration of a non-Tac peptide that binds interleukin 2: a potential participant in a multichain interleukin 2 receptor complex.
Proc. Natl. Acad. Sci. U.S.A. 83:9694-8

Tsudo, M., Kozak, R.W., Goldman, C.K. and Waldmann, T.A. (1987a) Contribution of a p75 interleukin-2 binding peptide to a high-affinity interleukin-2 receptor complex.
Proc. Natl. Acad. Sci. USA 84:4215-8

Tsudo, M., Goldman, C.K., Bongiovanni, K.F., Chan, W.C., Winton, E.F., Yagita, M., Grimm, E.A. and Waldmann, T.A. (1987b) The p75 peptide is the receptor for interleukin 2 expressed on large granular lymphocytes and is responsible for the interleukin 2 activation of these cells.
Proc. Natl. Acad. Sci. U.S.A 84:5394-8

Tysk, C., Lindberg, E., Jarnerot, G. and Floderus-Myrhed, B. (1988) Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking.
Gut 29:990-996

Underdown, B.J., De Rose, J. and Plaut, A. (1977) Disulfide bonding of secretory component to a single monomer subunit in human secretory IgA.
J. Immunol. 118:1816-1821

Van Hees, P. A. M., Van Elteran, P. H., Van Lier, H. J. J. and Van Tongeren, J. H. M. (1980) An index of inflammatory activity in patients with Crohn's disease.
Gut 21:279-286

Verspaget, H. W., Pena, A. S., Weterman, I. T., Lamers, C. B. H. W. (1988) Disordered regulation of the in vitro immunoglobulin synthesis by intestinal mononuclear cells in Crohn's disease.
Gut 29:503-510

Vives, J., Parks, D.E., and Weigle, W.O. (1980) Immunologic unresponsiveness after gastric administration of human gamma-globulin: antigen requirements and cellular parameters.
J. Immunol. 125(4):1811-6

Voitk, A.J., Enchave, B., Feller, J.H.,Brown, A. R. and Gurd, F. N. (1973b) experience with elemental diets in the treatment of inflammatory bowel disease. Is this primary therapy?
Arch. Surg. 107:329-333

Wakefield, A.J., Sawyerr, A.M., Dhillon, A.P., Pittilo, R. M., Rowles, P. M., Lewis, A. A. M. and Pounder, R. E., (1989) Pathogenesis of Crohn's disease: multifocal gastrointestinal infarctions.
Lancet ii:1057-62

Walker-Smith, J.A., Benfield, G.A., Montgomery, R.D. et al. (1986) Chronic inflammatory bowel disease in immigrants in the United Kingdom: in Frontiers of Gastrointestinal Research: The genetics and epidemiology of inflammatory bowel disease Vol 11 (eds R.McConnell, P. Rozen and T. Gilat), Karger, Basel, pp 18-25

Wang, Huey-Mei. and Smith, K.A. (1987) The interleukin 2 receptor. Functional consequences of its bimolecular structure.
J. Exp. Med. 166:1055-69

Wensick, F., Van de Merwe, J.P. and Mayberry, J.F. (1983) An international study of agglutinnins to Eubacterium, Peptostreptococcus and Coprococcus species in Crohn's disease, ulcerative colitis and control subjects.
Digestion 27:63-9

Werner, I. (1949) On the regeneration of serum proteins in normal and intoxicated rabbits.
Acta. Physiol. Scand. 19:27

Whorwell P.J., Holdstock, G., Whorwell, G.M. and Wright, R. (1979) Bottle feeding, early gastroenteritis and inflammatory bowel disease.
Br. Med. J. i:382

Wiman, K., Curman, B., Forsum, U., Klareskog, L., Malmnäs-Tjernlund, U., Rusk, L., Trägårdh. and Peterson, P.A. : (1978) Occurrence of Ia antigen on tissues of non-lymphoid origin.
Nature 276:711-713

Winitz, M., Graff, J., Gallagher, N., Narkin, A. and Seedman, D. A. (1965) Evaluation of chemical diets as nutrition for man-in-space.
Nature 4973:741-743

Wirostko, E., Johnson, L. and Wirostko, B (1987) Crohn's disease. Rifampin treatment of the ocular and gut disease.
Hepatogastroenterology 34(2): 90-3

Workman, E., Alun Jones, V., Wilson, A.J. and Hunter, J.O. (1984). Diet in the management of Crohn's disease.
Hum. Nut. Appl. Nut 38A:469-473

Wright, J.P., Alp, M.N., Young, G.O. and Tigler-Wybrandi, N. (1987) Predictors of acute relapse of Crohn's disease: A laboratory and clinical study.
Dig. Dis. and Sci. 32:164-170

Wu, K.C., Mahida, Y.R., Priddle, J.D. and Jewell, D.P. (1989) Immunoglobulin production by isolated intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease.
Clin. Exp. Immunol. 78:37-41

Wu, S.G. and Miyamoto, T. (1990) Radioprotection of intestinal crypts of mice by recombinant Interleukin-1 alpha.
Radiat. Res. 123:112-115

WÜthrich, B., Joller-Jemelka, H., Helfenstein, U. and Grob, P. J. (1990) levels of soluble interleukin-2 receptors correlate with the severity of atopic dermatitis.
Dermatologica 181:92-7

Yamada, T., Fujishima, A., Kawahara, K., Kato, K. and Nishimuras T. (1987) The importance of disulfide linkage for constructing the biologically active human interleukin-2.
Arch. Biochem. Biophys. 257:194-9

Zeitz, M., Green, W.C., Peffer, N.J. and James, S.P. (1988) Lymphocytes isolated from the intestinal lamina propria of normal non-human primates have increased expression of genes associated with T-cell activation.
Gastroenterology 94:647-655